INTEGRATED TAXONOMY AND MOLECULAR PHYLOGENY OF CONIDAE (MOLLUSCA: GASTROPODA) OF THE INDIAN COAST

FINAL SYNOPSIS OF THE THESIS

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Ph.D Thesis -Title

"Integrated Taxonomy and Molecular Phylogeny of Conidae (Mollusca: Gastropoda) Of the Indian Coast"

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Chapter 1: Introduction and review of literature

1.1 Cone snails

The marine molluscan family Conidae, Fleming 1822, represents a diverse group of invertebrate species and are well known for their evolutionary diversification, their unique neurotoxic venom peptides, and the beauty of their various shells. The currently accepted number of cone snail species stands at more than a thousand (WoRMS, 2022). They include such noted species such as the 'Glory-of-the-India' Cone (*Conus milneedwardsi*) and the deadly Geography Cone (*Conus geographus*). The superfamily conoidea comprises three significant families, including the best known and most intensively studied, the Conidae, along with the two other groups of venomous gastropods, the terebridae and the turrids. Cone snails live in the world's tropical and subtropical seas, from the intertidal to depths of almost 1,000 m (Kohn and Perran, 1994; Tenorio *et al.*, 2020).

1.2 Cone snails in India

The taxonomy and distribution of the diversity of cone snails in Indian Coastal waters is documented by (Kohn 1978). Various studies on the taxonomy and distribution of Conidae along the Indian Coasts carried out during 1835–2007 have recorded about 77 species. Most of these

were reported from the Gulf of Mannar, South east coast (Kohn 2001; Röckel *et al.* 1995; Franklin *et al.* 2007). Thus, the diversity and distribution of Conidae found in the coastal waters of India, particularly of Tamil Nadu, in the southeast part of the country, has been documented (Franklin *et al.*, 2007). However, these studies were mainly based on sporadic samplings. Since there has been no long-term study on the Indian Conidae particularly to the Coral Islands and West Coast, the present study is to observe the species of *Conus* distributed along the Indian coast.

1.3 Molecular markers and Species level molecular phylogeny of cone snails

Species delimitation using morphological data alone is difficult in molluscs because the presence of taxonomic ambiquity and species complex due to lack of clear morphological distinctiveness Recently tested hypotheses about the origin and evolution of conesnails feeding ecology, and radula modifications and examined the evolutionary history of Conus and reconstructed conidae phylogenies from mitochondrial and nuclear markers of Indo Pacific species of Conus(Duda and Kohn 2005; Duda *et al*., 2008) . Previous studies clearly describes molecular sequence data are a powerful tool for delimiting species, particularly in cases of hyper diverse groups where morphological differences are obscure. Distinguishing species in the Cone snail complex of tropical marine gastropods has long been difficult, because descriptions and identification has relied exclusively on shell characters, primarily colour patterns, and these often appear to intergrade among putative species (Pulliadre *et al.*, 2014).

OBJECTIVES

- To inventorise and establish taxonomic status of Cone snail (Mollusca) in Indian waters.
- To establish integrated (morphological and molecular) taxonomic identities for the Cone snails in Indian water.
- To generate species specific molecular signatures using mitochondrial genes (CO-1, 12sRNA, 16sRNA), nuclear gene (H3) and elucidate thylogenetic relationship for the target group of species.

Chapter 2. Diversity and morphological taxonomy of Indian conidae with the description of a new species *Conus laccadivensis* sp. nov from the Lakshadweep archipelago

2.1 Introduction

The Indian Ocean consists of a wide range of ecosystems namely coral reefs, mangroves, sea grasses and intertidal rocky shores. These niches harbor diverse species, including molluscan fauna (Kohn 2001, Ravinesh and Kumar, 2015; Ravinesh et al., 2018) with a wide range of archipelagic endemism. The taxonomy and distribution of the diversity of cone snails in Indian Coastal waters is fairly well documented by Kohn 1978. Various studies on the taxonomy and distribution of Conidae along the Indian Coasts carried out during 1835–2007 have recorded about 77 species. Most of these were reported from the Gulf Mannar, South east coast (Kohn 2001; Röckel *et al.* 1995; Franklin *et al.* 2007).

Thus, the diversity and distribution of Conidae found in the coastal waters of India, particularly of Tamil Nadu, in the southeast part of the country, has been documented (Franklin *et al.*, 2007). However, these studies were mainly based on sporadic samplings. Since there has been no long-term study on the Indian Conidae particularly in the Coral Islands and West Coast, the present study is to understand the distribution and diversity of the species of Conus along the Indian coast.

2.2 Methodology

2.2.1 Sample collection

A total of 50 species (255 individuals) from the families Conidae were collected from sampling sites along the both coasts of India including Gul of Kutch, Gul of Mannar, Andaman Nicobar and Lakshadweep Archipelagos. All the specimens were collected by snorkelling or SCUBA (Fig 2.1). Tissue samples from the region of muscular foot were taken and preserved in 95% ethanol untill further use.

2.2.2 Morphological Analysis

Specimens were identified based on shell morphology; morphometric and meristic characteristics and were recorded based on Rockel et., al 1995.

Fig 2.1 Sample collection, Kalpeni, Lakshadweep

2.3 RESULT

A total of 50 species (255 individuals) from the families Conidae were examined for morphological parameters, Shell color is noted initially and meristic characters such as Standard

Length (SL) Shell Width (SW); Aperture Height (AH); Relative diameter (RD) Relative spire height (RSH); Position of the maximum diameter (PMD) were recorded for the all the individuals.

***Conus laccadivensis* sp. nov.**

Five specimens of *Conus laccadivensis* sp. nov., were examined for morpho-meristic characters and successfully sequenced for fragments of the COI, 12S and 16S rRNA genes.

Systematics

Superfamily **Conoidea** Fleming 1822

Family **Conidae** Fleming 1822

Genus ***Conus*** Linnaeus, 1758

***Conus laccadivensis* sp. nov.,**

Type material: Holotype. The holotype (Fig 2.2 A) was deposited in the national repository Museum collections of Marine Biodiversity Museum, Central Marine Fisheries Research Institute, (holotype accession number DB.35.1.26) Cochin, Kerala, India.

Additional material: *Conus distans* Hwass, 1792 MNHN-IM-2007-30646 Standard Length 70.2 mm (Fig. 2.2. C) and MNHN-IM-2007-30833 Standard Length 46.7 mm (Fig. 2.2 D)

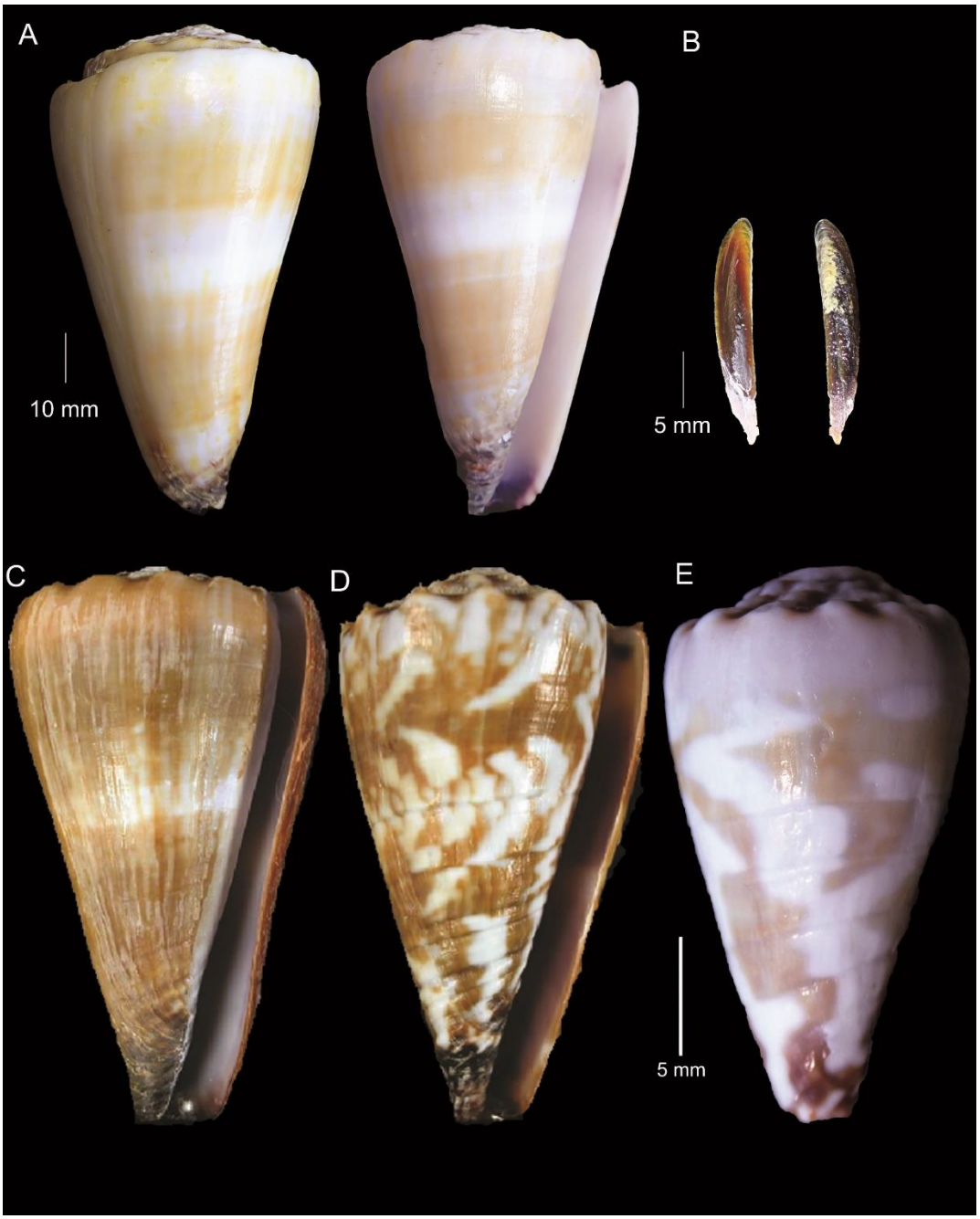

Fig 2.2. Holotype specimen of *Conus laccadivensis* sp. nov., CMFRI MBD C2.1 (A), operculum of *C. laccadivensis* (B), *Conus distans* Hwass, 1792 MNHN-IM-2007-30646 (C),

2.4 Discussion

The marine gastropod family Conidae is a hyperdiverse group of marine gastropods, and the primary species identification is based on shell morphology. In the present study, we collected 50 species of live specimens of cone snails and identified them along the Indian coast, including the remote Lakshadweep and Andaman & Nicobar archipelagos. The plasticity of morphological characters is common in cone snails (Duda and Kohn 2005). The use of complex potent neurotoxic venoms by cone snails to catch their prey is getting more attraction from pharmacologists. These venoms are playing a significant role in neurological and other drug development. Prior studies reported 84 species of Conidae from India (Franklin et al.,2007) including 49 of those collected in this study. Our study has increased the number of species of Conidae by describing a new species from the Lakshadweep archipelago. *Conus laccadivensis* sp nov.is morphologically similar to the Pacific species *C. distans* but with a very distinct color pattern and with large and closely spaced coronations.

Chapter 3. Multiple molecular markers based phylogeny of Indian conidae

3.1 Introduction

Cone snails are ideal organism for evolutionary genetic and biogeographic studies on molluscs because of short species divergence time (Kohn, 1990; Duda and Kohn, 2005; Duda et al., 2008) and its exceptional diversity of more than thousand widely distributed throughout tropical and sub-tropical Oceans, high degree of endemism and varied depth distributions (Tenorio et al., 2020). The marine species undergoes genetic changes through accumulation of mutations and genetic drifts which are subjected to selection pressure (Palumbi 1994) Duda and Palumbi (1999), Duda *et al*., (2008) reconstructed phylogenies from mitochondrial and nuclear gene sequences of 75 largely Indo Pacific species of Conus. They tested hypotheses about the evolution of life histories, feeding modes, and radulas, and roughly examined the evolutionary history of Conus. Duda *et al.,* 2008 clearly describes molecular sequence data are a powerful tool for delimiting species, particularly in cases of hyper diverse groups where morphological

differences are obscure. The present study is the first time approach for generating species-specific molecular signatures for India cone snails using both mitochondrial and nuclear markers.

3.2 Methodology

3.2.1 Sample collection

Samples were collected from the sampling sites along the coast of India. Tissue samples from the region of mantle were taken and preserved in 95% ethanol till further use. Specimens were analyzed based on shell morphology and morphometric characteristics were recorded.

3.2.2 DNA extraction and PCR amplification

DNA was isolated from up to 25 mg of foot tissue using customized Origin marine animal kit, premium according to the manufacturer's instructions was used and the DNA isolation was standardized to obtain optimum quality DNA. The quality and quantity of DNA were tested by Nanodrop (Eppendorf pvt. Ltd).1µl (50-75ng) of DNA from each genomic extraction was used as template for *in vitro* amplification using polymerase chain reaction (PCR). The PCR amplification reactions were performed in a total volume of 25 µl and included master mix (Orion, Origin Premium) in a DNA Engine Thermal Cycler under the appropriate conditions. Fragments of the mitochondrial genes 12S rRNA, 16S rRNA and cytochrome oxidase subunit I (COI) gene were amplified using universal primers (Table 3.1). All PCRs were performed in 25 ml, containing 75 ng of, 2.5 mM MgCl2, , 0.3 mM each primer. Amplification reaction consisted of an initial denaturation step at 94ºCfor 4 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing for 40 s at 54ºC, 52 ºC,50ºC and 52ºC for 12S, 16S COI and H3 genes respectively, and extension at 72ºC for 1 min. The final extension was at 72ºC for 5 min. PCR products were electrophoresed and visualized on 1.2 % agarose gels containing ethidium bromide.

3.2.3 Sequencing and Phylogenetic analysis

Specimens are registered in the MBD collections and sequences were deposited in GenBank . COI sequences of cone snails were downloaded from GenBank and aligned with the newly produced COI sequences using using BIOEdit (Hall 1999). A maximum likelihood (ML)

analysis with Kimura-2-parameters (K2P) genetic distances was performed using MEGA 7 (Kumar *et al.*, 2016) to roughly identify the genetic

Table 3.1 PCR Primers used in the present study

Gene	Primer	Sequence	Reference	Product size obtained
COI	LCOI	5-CGTCAACAAATCATAAAGATATTGG-3	Folmer et.al (1994)	658bp
	HCOI	5-TAAACTTCAGGGTGACCAAAAAATCA-3		
16S rRNA	16Sar	5-CGCCTGTTTATCAAAAACAT-3	Palumbi et al., 1996	510bp
	16Sbr	5-CCGGTCTGAACTCAGATCACGT-3		
12S rRNA	12SF	5-AAAGCTTCAAACTGGGATTAGATACCCCACTAT-3	Simon *et al.*, 1991	420bp
	12SR	5-TGACTGCAGAGGGTGACGGGCGGTGTGT-3		
H3	H3A	5-ATG GCTCGTACCAAGCAGACAGC-3	Colgan et al (1998)	325bp
	H3B	5-ATATC CTTRGGCATRATRGTGAC-3		

3.3 Result

The average sequence length of COI (658 bp), 16S (500 bp) and 12S (450 bp) were obtained from the five specimens of *Conus laccadivensis* sp. nov., Analyses of the partial mitochondrial COI sequence revealed well differentiated clades for C. *laccadivensis* sp. nov., and *C. distans* partial 16S rRNA and 12S rRNA gene sequences resulted in phylogenetic trees with identical topology .

Maximum likelihood clustering approaches were done based on mitochondrial genes, Cytochrome oxidase I (COI) and 16S ribosomal RNA (16S rRNA) genes. Multiple molecular

markers based analyses revealed that haplotype variations are giving new insights into the evolutionary genetic process hidden in ecological speciation of molluscs. We also observed that evolution takes place in protein codin mitochondrial and nuclear markers at different pace. . Maximum likelihood model based phylogentic tree is constructed for all the 51 species based on mitochondrial genes, Cytochrome oxidase I (COI) and 16S ribosomal RNA (16S rRNA) genes.

Kimura 2 parameter K2P based evolutionary genetic analysis were done for all the species. K2P genetic distance for intra species level is 0 to 0.02 for protein coding genes and 0 to 0.01 for ribosomal RNA. Minimum inter species geneteic distance in COI is 0.04 and 0.025 in ribosomal RNA also observed. The intra-species K2P genetic difference among the five specimens of *Conus laccadivensis* sp. nov., ranged from 0 to 0.7% for COI and 0 to 0.2% for 16S and 12S rRNA genes, whereas inter-species difference with *Conus distans* was 7 to 7.6% for COI and 2.5 to 2.8% in 12S rRNA and 16S rRNA genes which is more than the threshold limit for inter-species genetic distances (> 6% in COI and > 2% in 16S, 12SrRNA) in Conidae. Fig 3 Maximum Likelihood (ML) phylogenetic tree obtained with the combined 16S and 12S rRNA genes.

3.4 Discussion

The *p*-distance of all the three genes between *C. laccadivensis* sp. nov., and *C. distans* was more than the threshold genetic distances generally considered as interspecific distances used for species delimitation and validation in this hyperdiverse cone snails (Duda *et al.*, 2008; Puillandre *et al.*, 2011; Puillandre *et al*., 2014 and Abalde *et al.*, 2017). Ecological adaptive radiation based approaches have revealed that divergent venoms are evolving in cone snails with speciation and a novel species might produce modified peptides (Duda et al., 2008; Duda and Palumbi 1999). Speciation is promoted by divergent natural selection in many taxa including *Conus* (Kohn 1990; Palumbi 1994; Duda and Palumbi 1999). However, geographic and genetic factors have been affecting the process of speciation (Palumbi 1994). Genetic analysis of rapidly evolving mitochondrial coding (COI) genetic markers, slowly evolving non-coding (12S rRNA, 16S rRNA) genetic markers along with nuclear markers are thus ideal tools for understanding the evolutionary mechanism of speciation.

Chapter 4. Integrative taxonomy reveals that all the phenotype variations are not underpinned by genome in conidae (gastropoda, mollusca)

4.1 Introduction

High levels of shell morphological diversity can be seen in marine mollusks. Phenotypic changes can result from cumulative genetic variety brought on by the discontinuity of gene flow among populations as well as from a reaction to changing environmental variables (Palumbi 1994;). Morphological variations associated with adaptation enhance the survival rate of individuals. Phenotypic plasticity in marine gastropods can arise from two kinds of factors, either adaptive as in modular changes in growth, development and behavior in response to environmental cues) or non-adaptive as in stressful environments or poor diets resulting in slow growth or low survival. Additionally, plasticity may encourage the emergence of novel phenotypes, divergence among populations and species, and adaptive radiation (Beger et al., 2014).

Cone snails are the ideal organisms for studying the global challenges and conservation-based studies on molluscs because of their exceptional diversity of more than a thousand species (WoRMS 2022) widely distributed throughout the tropical and sub-tropical oceans, high degree of endemism and varied depth distribution.

4.2 Material and methods

4.2.1 Sample collection

Samples were collected from three sampling sites along the South East coast of India. Tissue samples from the region of muscular foot were taken and preserved in 95% ethanol till further use. Specimens were analyzed based on shell morphology and Morphometric characteristics were recorded.

4.2.2 DNA extraction and PCR amplification

DNA was isolated from up to 25 mg of foot tissue using customized Origin marine animal kit, premium according to the manufacturer's instructions was used and the DNA isolation was standardized to obtain optimum quality DNA. The quality and quantity of DNA

were tested by Nanodrop (Eppendorf pvt. Ltd).1µl (50-75ng) of DNA from each genomic extraction was used as template for *in vitro* amplification using polymerase chain reaction (PCR). The PCR amplification reactions were performed in a total volume of 25 µl and included master mix (Orion, Origin Premium) in a DNA Engine Thermal Cycler under the appropriate conditions.Fragments of the mitochondrial genes 12S rRNA, 16S rRNA and cytochrome oxidase subunit I (COI) gene were amplified using universal primers(Table 1). All PCRs were performed in 25 ml, containing 75 ng of, 2.5 mM MgCl2, , 0.3 mM each primer. Amplification reaction consisted of an initial denaturation step at 94ºCfor 4 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing for 40 s at 54ºC, 52 ºC,50ºC and 52ºC for 12S, 16S COI and H3 genes respectively, and extension at 72ºC for 1 min. The final extension was at 72ºC for 5 min. PCR products were electrophoresed and visualized on 1.2 % agarose gels containing ethidium bromide.

4.2.3 Sequencing and Phylogenetic analysis

Specimens are registered in the MBD collections and sequences were deposited in GenBank . COI sequences of cone snails were downloaded from GenBank and aligned with the newly produced COI sequences using using BIOEdit (Hall 1999). A maximum likelihood (ML) analysis with Kimura-2-parameters (K2P) genetic distances was performed using MEGA 7 (Tamura *et al.* 2016) to roughly identify the genetic

4.3 Result

Specific differences were observed for shell coloration. Maximum likelihood clustering analysis based phylogenetic tree were constructed for three mitochondrial genes, COI (Fig. 4.1), combined 16s rRNA , 12s rRNA and Nuclear H3 gene using five specimens each of varying phenotypes of targeted species. The analysis revealed that the phenotypic variations in *C. inscriptus C.amadis and C.malacanus* in different environments are not underpinned by the genome. K2P pair wise analysis based COI sequence shows the intra specific variation up to 0.0 to 0.5. Combined mitochondrial 16SrRNA and 12SrRNA shows small genetic variation compared with COI. Nuclear histone H3 also showing the same pattern of diversification non-coding mitochondrial 12SrRNA and 16S rRDNA. Present study reveals the all the phenotypic variations are not underpinned by genome.

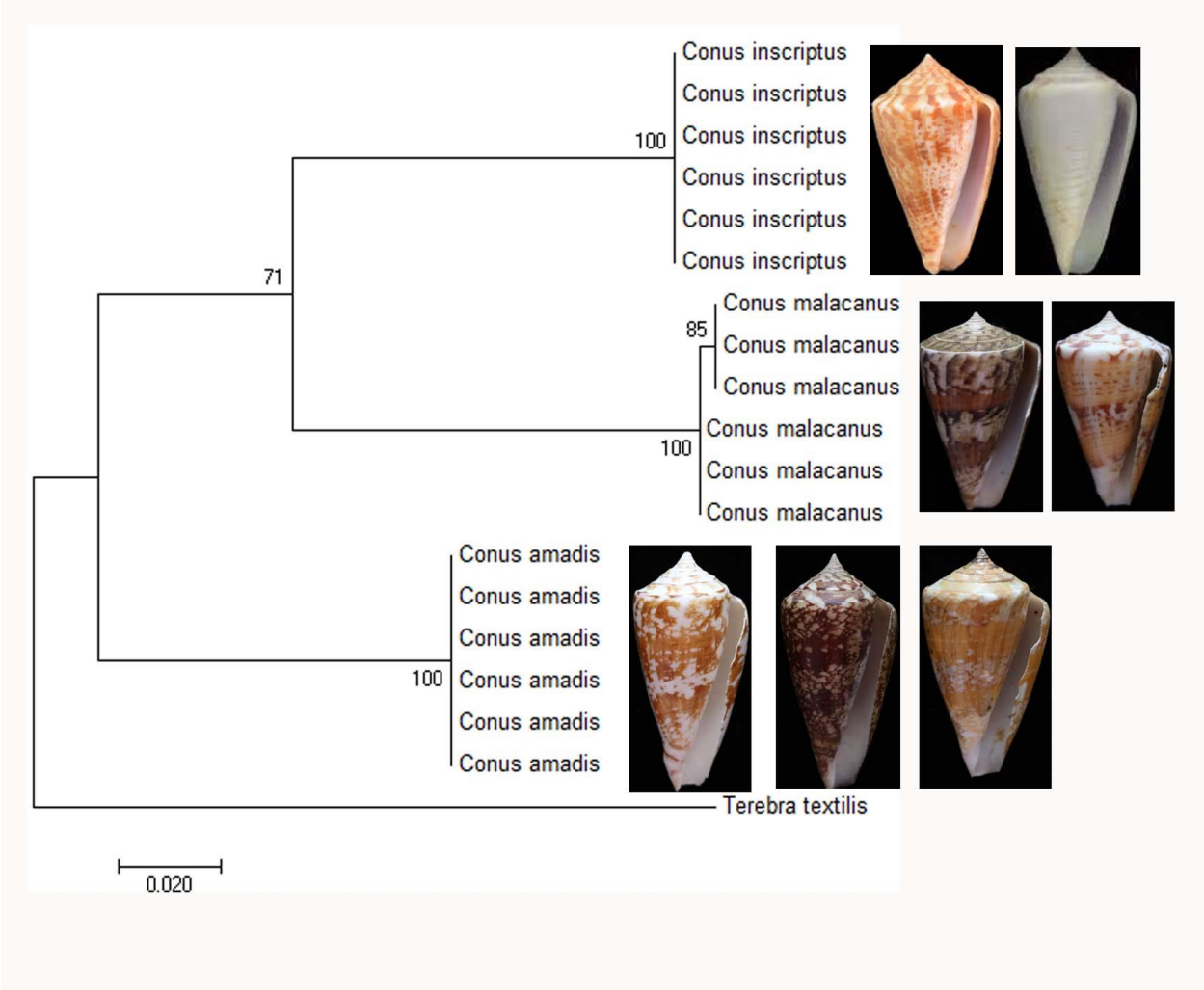

Fig. 4.1.Molecular Phylogenetic analysis by Maximum Likelihood method (Mitochondrial COI)

The different gene regions differ in their ability to resolve relationships among members. The rapid rate of gene evolution in Conus had led us to predict that noncoding mitochondrial 16S and 12S sequences would detect genetic differences among evolutionarily distinct units more clearly than mitochondrial COI.

4.4 Discussion

In Conidae species delimitation using morphological data is very difficult because the presence of species complex due to lack of clear morphological distinctiveness and existence of morphological intergradation among described species, *C.flavidus, C. frigidus* and their synonyms *C.peasi* with slight morphological variation into valid species was revealed by using genetic analysis (Lawler and Duda 2017). Similarly *C.conco* and *C. lividus* with slight morphological variation, the integrative approaches revealed *C.conco* as novel species (Puillandre et al 2014,) Molecular markers based species delimitation were did in the *C. sponsalis* complex, another complex in the conus genus where morphological characters alone were unable to resolve the taxonomic ambiguity (Duda *et al*. 2008).

We analysed different morphotype specimens of same species for genetic analysis, present study developed the first species specific molecular signature for *C.malacanus and C.amadis* *C. malacanus* is similar to *C. sukhadwalai* and sometimes also to *C. lenavati*. Subadult specimens of *C. malacanus* may be confused with *C. eximius*. Present study also provided the molecular based confirmation of invalidity of holotype of *C. cuneatus* , which is a subadult specimen of *C. malacanus* with reduced pattern**.** These findings will help the conservation and management of these species, same genetic stock can be managed as a single unit.

Chapter 5. Integrated taxonomy reveals the presence of cryptic speciation in *Conus varius* and *C. litteratus* endemic to the Arabian Sea and Pacific ocean

5.1 Introduction

The Arabian Sea, Indian Ocean harbors a wide range of marine ecosystems and the endemic biodiversity in the Arabian Sea is strongly linked to many unique ecological and biogeographical features (Laxmilatha et al., 2021). Studies on biogeographical factors reveal the discontinuities in species spatial distribution and genetic diversity of species. Major barriers which restrict larval distribution and gene flow between oceanic macro ecosystems have previously been identified (Beger et al., 2014). New Insights into the mechanism of larval dispersal is the road map of evolutionary studies in the oceanic ecosystem and is considered an essential component of gene

flow (Palumbi, 1994; Beger et al., 2014). Other different ecological and evolutionary genetic processes can also operate within populations, resulting in inter-species level genetic diversity (Mayr, 1954; Wainwrigh et al., 2020).

The west coast of India is one of the highly productive seas in the Indian Ocean region due to its boundaries and the oceanographic processes, with strong seasonal upwelling resulting in high levels of primary productivity that shapes unique marine biodiversity in the eastern Arabian Sea including Lakshadweep archipelago.

5.2 Material and Methods

5.2.1 Sample collection

Specimens were collected from the lagoon of Agatti Island of Lakshadweep archipelago of Arabian Sea by snorkelling. Tissue samples from the foot region were taken and preserved in 100% ethanol until further use.

5.2.2 DNA extraction and PCR amplification

DNA was isolated from foot tissue using customized origin marine animal kit, premium and used according to the manufacturer's instructions. The quality and quantity of DNA were tested by Nanodrop (Eppendorf Pvt. Ltd). Fragments of the mitochondrial gene cytochrome oxidase subunit I (COI) gene was amplified using universal primers LCO1490/HCO2198 (Folmer *et al*., 1994). All PCR reactions were performed in 25 µl, containing 75 ng of DNA, 2.5 mM $MgCl_2$, 0.3 mM each primers. Amplification reaction consisted of an initial denaturation step at 94ºC for 4 min, followed by 30 cycles of denaturation at 94ºC for 30 s, annealing for 35 s at 50ºC and extension at 72ºC for 1 min. The final extension was at 72ºC for 5 min. All PCR products were electrophoresed and visualized on 1.2 % agarose gels containing ethidium bromide.

5.2.3 Sequencing and phylogenetic analysis

We obtained COI, sequences of Pacific specimens and closely related species from the GenBank, NCBI database and aligned with the newly generated sequences using the BIO Edit (Hall, 1999).A maximum likelihood (ML) analysis with Kimura-2-parameters (K2P) genetic distances was performed using MEGA 7 (Kumar 2016) to identify the group in which Arabian sea and Pacific specimen clustered.

5.3 Result

Morphological parameters were noted for both the species. *C. varius* standard length 109 mm; shell width 63 mm; aperture height 94 mm; relative diameter 0.062mm; relative spire height 0.12 mm; position of the maximum diameter 0.88 mm; and for *C.littretus* standard length 109 mm; shell width 63 mm; aperture height 94 mm; relative diameter 0.062mm; relative spire height 0.12 mm; position of the maximum diameter 0.88 mm.

Analyses of the partial mitochondrial COI sequence revealed well differentiated clades for C.*varius* (Fig 5.1) and *C. littretus*. The intra-species K2P genetic difference among the specimens of *C.varius* ranged from 4 to 4.5 % for COI (Table 5.1)whereas inter-species difference with *Conus littraus* was 5 to 6 % for COI

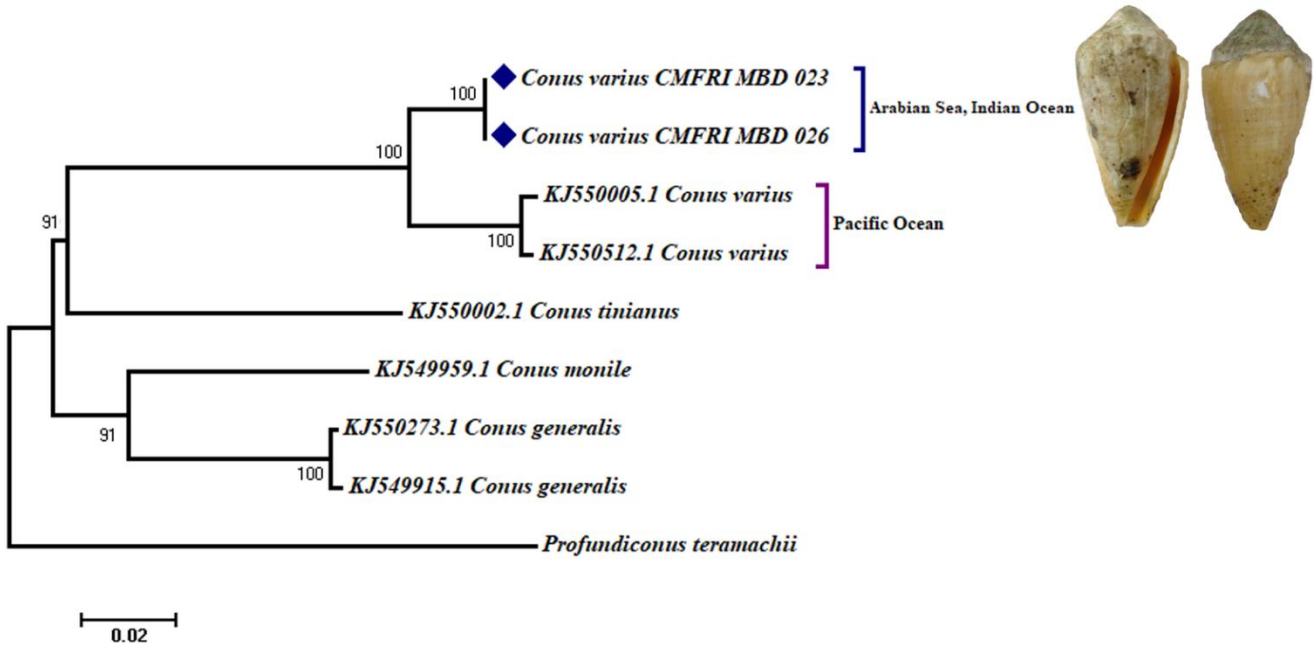

Fig. 5.1 Phylogenetic tree based on COI gene representing genetic variant of *C.varius* of Arabian Sea and Pacific Ocean

Table 5. 1 Kimura 2 parameter (K2P) genetic distance

Conus_varius_CMFRI_MBD_023 (Arabian Sea)								
Conus_varius_CMFRI_MBD_026 (Arabian Sea)	**0.000**							
KJ550005.1_Conus_varius* (Pacific*)	**0.040**	**0.040**						
***KJ550512.1_Conus_varius (*Pacific)**	**0.045**	**0.045**	**0.005**					
KJ550273.1_Conus_generalis	0.152	0.152	0.159	0.154				
KJ549915.1_Conus_generalis	0.152	0.152	0.159	0.154	0.003			
KJ549959.1_Conus_monile	0.159	0.159	0.172	0.165	0.094	0.098		
KJ550002.1_Conus_tinianus	0.159	0.159	0.172	0.170	0.137	0.141	0.141	
Profundiconus_teramachii	0.213	0.213	0.230	0.227	0.182	0.182	0.188	0.193

5.4 Discussion

In our analysis, two genetically distinct specimens were observed for *C.varius* and *C.littretus* of Pacific and Arabian sea. This was revealed by the DNA analysis and are not morphologically distinguishable. Molecular techniques are now more frequently employed in taxonomic analysis and are revealing numerous cases of cryptic species in all groups, including molluscs (Duda *et al*., 2008; Puilladre *et al.*, 2014). In several cases, morphologically indistinguishable entities have been recognized as separate species in molluscs.

This unusually broad distribution makes *C.littretus and C.varius* an excellent subject for examining patterns of biogeographic genetic connectivity of populations and patterns of species level diversification in the broad Indian and Pacific Ocean realm. A study to interpret patterns of gene flow similarly demonstrated a general absence of genetic structure in western and central Pacific populations of *C. ebraeus* by using mitochondrial gene sequences (Duda and Kohn 2005) These molecular information can provide clear evidence for genetic boundaries between cryptic species.

Chapter 6. Mitochondrial markers based analyses provides new insights into role of coral reef ecosystems in molluscan speciation

6.1. Introduction

Understanding the mechanism of larval dispersal is the fundamental unit of evolutionary studies in the aquatic ecosystem and is considered as an essential component in gene flow (Beger et al., 2014). Despite this generalization, different ecological and evolutionary genetic process can operate within populations resulting in genetic diversity (Mayr, 1954;_Palumbi, 1994; Wainwrigh et al., 2020). It is also recognized that evolution in the marine ecosystem may operate under a different set of rules, given the difficulties in applying allopatric models to marine ecosystem (Mora and Sale 2002). Geographical barriers are few in the ocean and planktonic larval dispersal is often extensive in marine invertebrates (Palumbi, 1994), a combination that should limit speciation. Yet, archipelagic ecosystems represent high species diversity indicating speciation mechanism beyond conventional allopatric models.

Mitochondrial and nuclear molecular markers based genetic data can address biogeographic evolutionary mechanisms in different ways. Variations in the sequence data can provide evidence for evolutionary genetic boundaries between cryptic species and information about the geography of haplotype variants within species (Palumbi, 1994). Oceanic archipelagos have long served as unique experimental settings for ecological speciation studies (Whittaker and Fernández, 2007; Losos and Ricklefs, 2009). The evolutionary genetic divergence of island populations and in particular the pace, tempo and relative adaptive radiations are well known (Aleixandre et al., 2013) and recent studies in Lakshadweep Islands also shows its speciation potential (Laxmilatha et al., 2021).

Specific differences were observed for shell coloration of *Conus catus* specimens collected from different ecosystems such as rocky intertidal beds, lagoons, and inner reef platforms of Lakshadweep (Arabian Sea) and Andaman and Nicobar (Bay of Bengal) archipelagos of Indian Ocean. The genotypic characterization of these ecotypes based on protein coding and non-coding mitochondrial markers were examined to understand whether they are under the selective pressure of ecological speciation.

6.2 Material and Methods

6.2.1 Sample collection

Specimens were collected from rocky intertidal shores, lagoons, and inner reef platforms of Lakshadweep (Arabian Sea) and Andaman and Nicobar (Bay of Bengal) archipelagos of Indian Ocean . Tissue samples from the mantle region were taken and preserved in 95% ethanol until further use. The morpho-meristic description of the shell was carried out following Rockel et al.(1995).

6.2.2 DNA extraction and PCR amplification

DNA was isolated from ethanol preserved tissue using Origin marine animal kit and used according to the manufacturer's protocol. The quantity and quality of DNA were tested by Nanodrop (Eppendorf Pvt. Ltd). Fragments of the mitochondrial 16S rRNA and cytochrome oxidase subunit I (COI) gene was amplified using universal primers 16Sar/16Sbr (Palumbi, 1996) and LCO1490/HCO2198 (Folmer et al., 1994), respectively. All PCR reactions were performed in 25 µl, containing 50ng of DNA, 3 mM $MgCl_2$, and 0.3 mM of each primer. Amplification reaction consisted of an initial denaturation step at 95ºC for 4 min, followed by 30 cycles of denaturation at 94ºC for 30 s, annealing for 30 s at , 53ºC, 48ºC for 16SrRNA and COI respectively, and extension at 72ºC for 1 min. The final extension was at 72ºC for 5 min. All PCR products were electrophoresed and visualized on 1.5% agarose gels containing ethidium bromide.

6.2.3 Sequencing and phylogenetic analysis

Amplified PCR products were sequenced using an ABI3730 capillary sequencer. Sequences were deposited in National Centre for Biotechnological Information (NCBI) data base and accession numbers are provided in Table 1. COI sequence (KJ550165) and 16S rRNA (KJ550812) sequence of the pacific specimen of *C. catus* was obtained from the NCBI data base. *Varioconus mercator* (MT240812, MT240813) was used as closely related outgroup and aligned with all sequences using the BioEdit (Hall, 1999). A maximum likelihood (ML) phylogenetic analysis with Kimura-2-parameters (K2P) model was performed using MEGA 7 (Kumar, 2016) to identify the group in which the *C. catus* specimens from different ecosystems are clustered.

6.3 Result

6.3.1 Morphological analysis

Morphological parameters were recorded for the *C. catus* species collected from different ecosystems viz; rocky intertidal beds, lagoons, and inner reef platforms of Lakshadweep and Andaman Nicobar archipelagos of Indian Ocean. Shells are moderately medium sized ranging from 25-28mm. Shell colour varied across the ecosystems; olive-brown, blackish brown in rocky shores and orange in sub tidal coral reef flats (Fig. 6.1). Relative diameter (RD) varies from 0.60 mm to 0.62 mm in rocky shores and 0.65 mm to 0.66 mm in sub tidal coral reef flats. Position of maximum diameter (PMD) varies from 0.65 mm to 0.66 mm in rocky shores and 0.77 mm to 0.79mm in sub tidal coral reef flats, while the relative spiral height (RSH) measured 0.10 mm to 0.11 mm and 0.12 mm to 0.13 mm respectively.

6.3.2. Molecular analysis

Maximum likelihood (ML) phylogenetic clustering analysis tree was constructed for mitochondrial genes, protein coding COI , and non coding 16s rRNA (Fig. 6.1) for the specimens of varying phenotypes of targeted species. The analysis revealed that the phenotypic variations in different environments are underpinned by the genome. Kimura 2 parameter (K2P) pair wise analysis based estimated genetic variation in COI sequence in between rocky shore and coral reef ecotypes are 0.010 to 0.011 (1 to 1.1 percentage), on the contrary rocky shore specimens of Lakshadweep and Andaman Islands showed genetic variation of 0.0 to 0.002 (0 to 0.02 percentage) and genetic distance between *C. catus* and *C. striolatus* are 0.108 to 0.112 (10.8. to 11.2 percentage). Mitochondrial 16S rRNA gene based estimated evolutionary genetic divergences between the rocky and coral specimens of *Conus catus* are 0.004 (0.4 percentage), while that of rocky shore specimens of Lakshadweep and Andaman Islands are 0.0 (0 percentage) and genetic distance between *C. catus* and *C. striolatus* are 0.040 to 0.045 (4.0 to 4.5 percentage)**.**

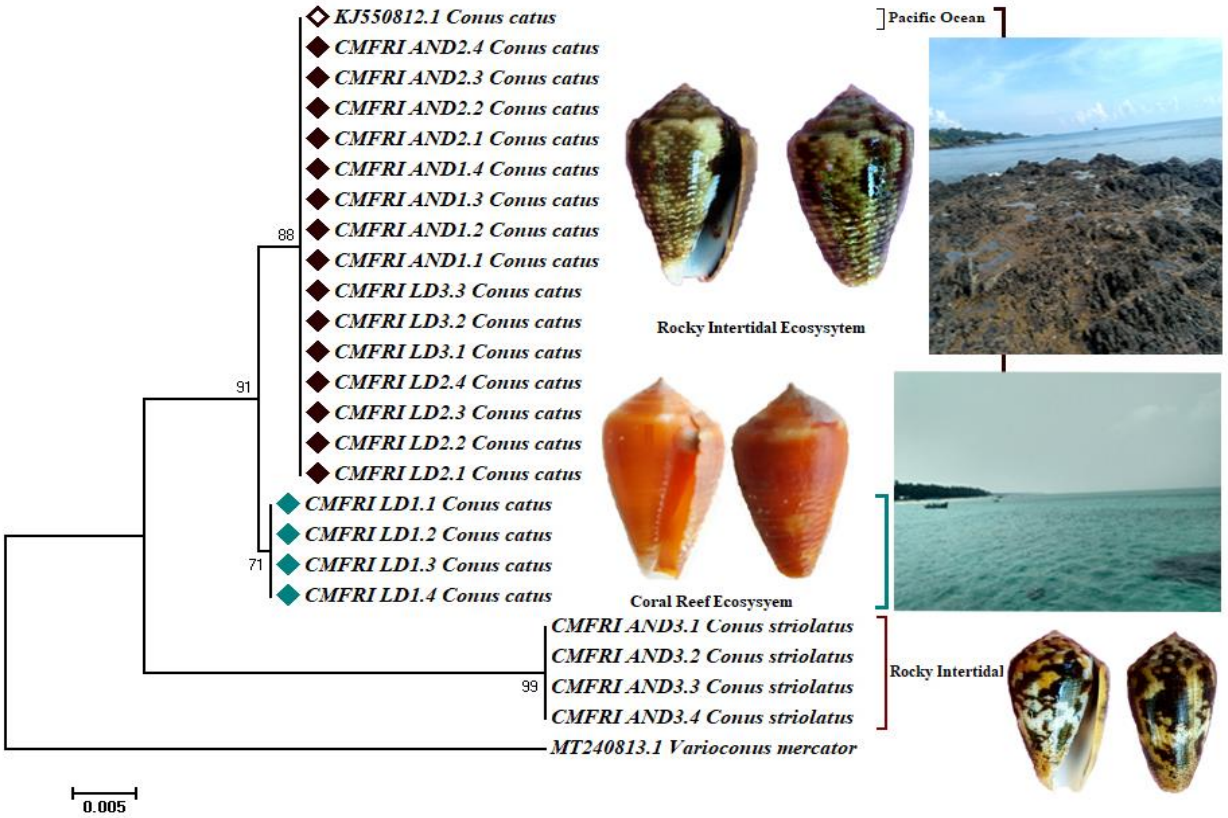

Fig. 6.1 Maximum likelihood (ML) phylogenetic tree constructed based on non coding 16s rRNA gene, representing ecologically and genetically different populations of *C. catus.*

Among the 19 aligned sequences of *C. catus*, coral reef population has major base substitution pattern at six base pair locations of COI and two base pair locations of 16S rRNA revealing the genetic difference in coral reef specimens. Genetic diversity indices analysed also confirmed population level divergence among coral and rocky groups. These COI sequences with 629 base pairs contained 622 monomorphic and seven polymorphic sites constituting three haplotypes. Coral reef haplotype included four individuals of coral reef population with six polymorphic sites. The 16S rRNA sequences with 465 base pairs contained two polymorphic sites constituting coral and rocky population haplotypes.

6.4. Discussion

Multiple mitochondrial molecular markers based analysis on ecotypes of *Conus catus* revealed that ecological niche depended divergent evolutionary selective pressure is acting on both mitochondrial protein coding rapidly evolving COI marker and non coding comparatively

slowly evolving 16S rRNA but in different paces. We observed molluscan phylogeographical patterns that contradict expectations of low genetic connectivity between similar habitats separated by thousands of kilometers and high genetic connectivity in adjacent and ecologically distinct habitats. Similarly mitochondrial DNA sequences based analysis in ecological speciation of reef fishes and shrimps with similar overlapping dispersal potential also contradict expectation of large geographical isolation and giving new insights into the role of nearby ecological distinct habitat in speciation (Rocha et al., 2005; Wainwrigh et al., 2020).

Previous studies suggest that adaptive divergence depended ecological speciation may happen despite opposition by gene flow and identifying the molecular basis of underlying genetic divergence with gene flow is a major task in evolutionary genetic studies (Schluter, 2001). Present study considered the archipelagic ecosystems in the different part of Indian Ocean as a natural laboratory and overcame the challenges like pelagic larval dispersal connectivity across the fluid medium which limits the understanding of the mechanism of ecological speciation (Palumbi, 1994; Jean, 2002).We compared the genetic variation of our rocky shore phenotype specimen with similar pacific specimen (Puilladre et al ., 2014) and observed the same genetic connectivity across the rocky shore habituated specimens regardless of the sea and oceans they belong.

Summary and Conclusions

Present study generated species specific molecular signatures for 255 individuals from 50 species of the families Conidae using mitochondrial genes (CO-1, 12srRNA,16srRNA) and nuclear H3 gene. This has been developed for the first time in the country which will be of immense use wherein there exists greater taxonomic ambiguity in Conus species for conservation and management purposes. The study described the cone snail, *Conus laccadivensis* sp. nov., based on the morphological and molecular characters of the materials collected from Kalpeni and Androth Islands of Lakshadweep Archipelago. The species closely resembles the tropical species *Conus distans*, (Hwass in Bruguière, 1792) which is widely distributed throughout the Indo-Pacific regions. Morphologically *C. laccadivensis* sp. nov., differs from *C. distans* in shell colour and spire characteristics. *Conus laccadivensis* sp. nov., possess distinct colour patterns with large and closely spaced coronations. The maximum likelihood clustering approaches using the mitochondrial genes such as COI, 16S rRNA and 12S rRNA revealed a separate genetic

cluster confirming the newly identified species. Integrative taxonomy reveals that all the phenotype variations are not underpinned by genome in conidae (gastropoda, mollusca) drastically varying phenotypes observed among the targeted Conus species of different environments using mitochondrial and nuclear markers.

Morphological parameters were recorded for all the specimens and varying the phenotypes of the species. Specific differences were observed for shell coloration. Three mitochondrial genes, COI (658bp), 16s rRNA (550bp), 12s rRNA (450bp) and Nuclear H3 gene (350bp) were amplified in five specimens each of varying phenotypes of targeted species. The analysis revealed that the phenotypic variations in *C. inscriptus C.amadis and C.malacanus* in different environments are not underpinned by the genome, The present study generated species-specific molecular signatures from varying phenotypes of conidae Since speciation is positively correlated with the complexity of conopeptides of the species, the findings provide confirmation of phenotypic variation and will be used for bioprospecting research on the species and also conservation and management of the species in Indian waters. In our analysis, two genetically distinct specimens were observed for *C.varius* and *C.litretus* of Pacific and Arabian Sea, revealed by the DNA analysis, are not morphologically distinguishable

This unusually broad distribution makes *C.littretus and C.varius* an excellent subject for examining patterns of biogeographic genetic connectivity of populations and patterns of species level diversification in the broad Indian and Pacific Ocean realm

Mitochondrial markers based analyses provides new insights into role of coral reef ecosystems in molluscan speciation. The high species diversity in tropical coral ecosystem indicate that it is driven by different set of mechanisms which are beyond conventional allopatric ecological speciation models, because geographical barriers are limited in marine ecosystems. Specific differences were observed in shell coloration of *Conus catus* (Hwass in Bruguière 1792), specimens collected from adjacent ecologically different habitats. Maximum likelihood clustering approaches were done based on mitochondrial genes, Cytochrome oxidase I (COI) and 16S ribosomal RNA (16S rRNA) genes.

Multiple molecular markers based analyses revealed that coral reef ecological niche depended adaptive divergent evolutionary selective pressure is acting on both coding and non-coding mitochondrial markers. Adjacent ecologically different habitats like rocky shore and sub

tidal coral reef flats in Lakshadweep Islands have less genetic connectivity compared with geographically separated similar ecosystems like rocky shores in Arabian sea, Bay of Bengal and Pacific Ocean. These haplotype variations are giving new insights into the evolutionary genetic process hidden in ecological speciation of molluscs. We also observed that *Conus striolatus* (Kiener 1845), a morphologically similar species to rocky shore *C. catus* is the outcome of sympatric speciation through the modified niche depended selection pressure exerting on each other in rocky intertidal ecosystems. The present study revealed that ecotypes of *C. catus* is in the way of parapatric speciation and reminds that basic unit of biodiversity conservation should be the habitat apart from the species.

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PUBLICATIONS

1) **Shijin Ameri., Laxmilatha; P.,** Labeeb, K. A., Ranjith L., Kathirvelpandian. (2021) A Mitochondrial marker–based analyses provide new insights into role of coral reef ecosystems in molluscan speciation. ***Organisms Diversity & Evolution***. https://doi.org/10.1007/s13127-021-00533-9.

2) **Laxmilatha, P**; **Shijin Ameri**.; Labeeb, K.; Ranjith, L.; Kathirvelpandian, A. (2021). A new species of cone snail, *Conus laccadivensis* sp. nov.: (Gastropoda: Conidae) from the Islands of Lakshadweep Archipelago. ***Regional Studies in Marine Science***. 44: 101783: 1-7., *available online at* https://doi.org/10.1016/j.rsma.2021.101783.

International Conference Presentations

1) **Shijin Ameri**., **Laxmilatha; P**., Labeeb, K. A., Ranjith L., Kathirvelpandian. A Integrated taxonomy of cone snails Giving new insight into the role of ecosystem in speciation , Abstract proceedings, Ocean sciences meet, OSM 2020 16-21 Feb 2020 San Diego CA USA. **(Received early career scientist grant by American Geophysical Union (AGU) and travel grant from British ecological society (BES)).**

2) **Shijin Ameri., Laxmilatha; P.,** Labeeb, K. A., Ranjith L., Kathirvelpandian. A Integrated taxonomy reveals the exceptional phenotypic plasticity in *Conus (Pinoconus) catus* among the Andaman and Lakshadweep archipelagos, Abstract proceedings, International conference on Benthos, ICB 6-8 March 2019 Kochi, India. **(Received best paper award).**

Pre print

1) ***Pappurajam, L., Ameri, S***., Labeeb, K. A., Kathirvelpandian, A., Ranjith, L., and Gopalakrishnan, A. (2018). Integrative taxonomy reveals that all the phenotype variations are not underpinned by genome in Conidae (Gastropoda, Mollusca). PeerJ Preprints 6: e26844v1 https://doi.org/10.7287/peerj.preprints.26844v1.

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ORIGINAL ARTICLE

Mitochondrial marker–based analyses provide new insights into role of coral reef ecosystems in molluscan speciation

Shijin Ameri[1] · **P. Laxmilatha**[1] · **K. A. Labeeb**[1] · **L. Ranjith**[2] · **A Kathirvel Pandian**[3]

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Abstract

The high species diversity in tropical coral ecosystem indicates that it is driven by different set of mechanisms which are beyond conventional allopatric ecological speciation models, because geographical barriers are limited in marine ecosystems. Specific differences were observed in shell coloration of *Conus catus* (Hwass in *Histoire naturelle des vers*. volume 1. Pancoucke, Paris, 1792), specimens collected from adjacent ecologically different habitats. Maximum likelihood clustering approaches were done based on mitochondrial genes, Cytochrome oxidase I (COI) and 16S ribosomal RNA (16S rRNA) genes. Multiple molecular marker–based analyses revealed that coral reef ecological niche–dependent adaptive divergent evolutionary selective pressure is acting on both coding and non-coding mitochondrial markers. Adjacent ecologically different habitats like rocky shore and subtidal coral reef flats in Lakshadweep Islands have less genetic connectivity compared with geographically separated similar ecosystems like rocky shores in Arabian sea, Bay of Bengal, and Pacific Ocean. These haplotype variations are giving new insights into the evolutionary genetic process hidden in ecological speciation of molluscs. We also observed that *Conus striolatus* (Kiener in *Icon. des Coq. Viv.*, 2, 1845), a morphologically similar species to rocky shore *C. catus*, is the outcome of sympatric speciation through the modified niche-dependent selection pressure exerting on each other in rocky intertidal ecosystems. The present study revealed that ecotypes of *C. catus* are in the way of parapatric speciation and remind that basic unit of biodiversity conservation should be the habitat apart from the species.

Keywords Parapatric speciation · Conidae · Genetic variation · Cytochrome oxidase I · 16S ribosomal RNA

Introduction

Understanding the mechanism of larval dispersal is the fundamental unit of evolutionary studies in the aquatic ecosystem and is considered an essential component in gene flow (Beger et al., 2014; Servedio & Noor, 2003). Despite this generalization, different ecological and evolutionary genetic processes can operate within populations resulting in genetic diversity (Mayr, 1954; Palumbi, 1994; Wainwright et al., 2020). It is also recognized that evolution in the marine ecosystem may operate under a different set of rules, given the difficulties with applying allopatric models to marine ecosystem (Mora & Sale, 2002). Geographical barriers are few in the ocean and planktonic larval dispersal is often extensive in marine invertebrates (Palumbi, 1994), a combination that should limit speciation. Yet, archipelagic ecosystems represent high species diversity indicating speciation mechanism beyond conventional allopatric models.

Mitochondrial and nuclear molecular marker–based genetic data can address biogeographic evolutionary mechanisms in different ways. Variations in the sequence data can provide evidence for evolutionary genetic boundaries between cryptic species and information about the geography of haplotype variants within species (Palumbi, 1994). Oceanic archipelagos have long served as unique experimental settings for ecological speciation studies (Losos & Ricklefs, 2009; Whittaker & Fernández-Palacios, 2007). The evolutionary genetic divergence of island populations and in particular the pace, tempo, and relative adaptive radiations are well known (Aleixandre et al., 2013), and recent studies in Lakshadweep Islands also show its speciation potential (Laxmilatha et al., 2021).

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Cone snails are ideal organism for evolutionary genetic and biogeographic studies on molluscs because of short species divergence time (Duda & Kohn, 2005; Duda et al., 2008; Kohn, 1990) and its exceptional diversity of more than 900 species (MolluscaBase., 2021) widely distributed throughout tropical and sub-tropical Oceans, high degree of endemism, and varied depth distributions (Tenorio et al., 2020).

Specific differences were observed for shell coloration of *Conus catus* specimens collected from different ecosystems such as rocky intertidal beds, lagoons, and inner reef platforms of Lakshadweep (Arabian Sea) and Andaman and Nicobar (Bay of Bengal) archipelagos of Indian Ocean. The genotypic characterization of these ecotypes based on protein coding and non-coding mitochondrial markers was examined to understand whether they are under the selective pressure of ecological speciation.

Material and methods

Sample collection

Specimens were collected from rocky intertidal shores, lagoons, and inner reef platforms (Table 1) of Lakshadweep (Arabian Sea) and Andaman and Nicobar (Bay of Bengal) archipelagos of Indian Ocean (Fig. 1). Tissue samples from the mantle region were taken and preserved in 95% ethanol until further use. The morpho-meristic description of the shell was carried out following Röckel et al. (1995).

DNA extraction and PCR amplification

DNA was isolated from ethanol preserved tissue using Origin marine animal kit and used according to the manufacturer's protocol. The quantity and quality of DNA were tested by Nanodrop (Eppendorf Pvt. Ltd). Fragments of the mitochondrial 16S rRNA and cytochrome oxidase subunit I (COI) gene were amplified using universal primers 16Sar/16Sbr (Palumbi, 1996) and LCO1490/HCO2198 (Folmer et al., 1994), respectively. All PCR reactions were performed in 25 µl, containing 50 ng of DNA, 3 mM $MgCl_2$, and 0.3 mM of each primer. Amplification reaction consisted of an initial denaturation step at 95 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing for 30 s at, 53 °C, 48 °C for 16SrRNA and COI respectively, and extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. All PCR products were electrophoresed and visualized on 1.5% agarose gels containing ethidium bromide.

Table 1 Details of specimens

Species	Specimen voucher	Location	Habitat	NCBI accession numbers	
				16S rRNA	COI
C. catus	CMFRI LD1.1	Agatti, Lakshadweep	Subtidal coral reef flats	MZ267084	MZ267116
C. catus	CMFRI LD1.2	Agatti, Lakshadweep	Subtidal coral reef flats	MZ267085	MZ267117
C. catus	CMFRI LD1.3	Agatti, Lakshadweep	Subtidal coral reef flats	MZ267086	MZ267118
C. catus	CMFRI LD1.4	Agatti, Lakshadweep	Subtidal coral reef flats	MZ267087	MZ267119
C. catus	CMFRI LD2.1	Agatti, Lakshdweep	Rocky shore	MZ267088	MZ267120
C. catus	CMFRI LD2.2	Agatti, Lakshadweep	Rocky shore	MZ267089	MZ267121
C. catus	CMFRI LD2.3	Agatti, Lakshadweep	Rocky shore	MZ267090	MZ267122
C. catus	CMFRI LD2.4	Agatti, Lakshadweep	Rocky shore	MZ267091	MZ267123
C. catus	CMFRI LD3.1	Kalpeni, Lakshadweep	Rocky shore	MZ267092	MZ267124
C. catus	CMFRI LD3.2	Kalpeni, Lakshadweep	Rocky shore	MZ267093	MZ267125
C. catus	CMFRI LD3.3	Kalpeni, Lakshadweep	Rocky shore	MZ267094	MZ267126
C. catus	CMFRI AND1.1	South Andaman	Rocky shore	MZ267095	MZ267127
C. catus	CMFRI AND1.2	South Andaman	Rocky shore	MZ267096	MZ267128
C. catus	CMFRI AND1.3	South Andaman	Rocky shore	MZ267097	MZ267129
C. catus	CMFRI AND1.4	South Andaman	Rocky shore	MZ267098	MZ267130
C. catus	CMFRI AND2.1	North Andaman	Rocky shore	MZ267099	MZ267131
C. catus	CMFRI AND2.2	North Andaman	Rocky shore	MZ267100	MZ267132
C. catus	CMFRI AND2.3	North Andaman	Rocky shore	MZ267101	MZ267133
C. catus	CMFRI AND2.4	North Andaman	Rocky shore	MZ267102	MZ267134
C. striolatus	CMFRI AND3.1	South Andaman	Rocky shore	MZ267103	MZ267135
C. striolatus	CMFRI AND3.2	South Andaman	Rocky shore	MZ267104	MZ267136
C. striolatus	CMFRI AND3.3	South Andaman	Rocky shore	MZ267105	MZ267137
C. striolatus	CMFRI AND3.4	South Andaman	Rocky shore	MZ267106	MZ267138

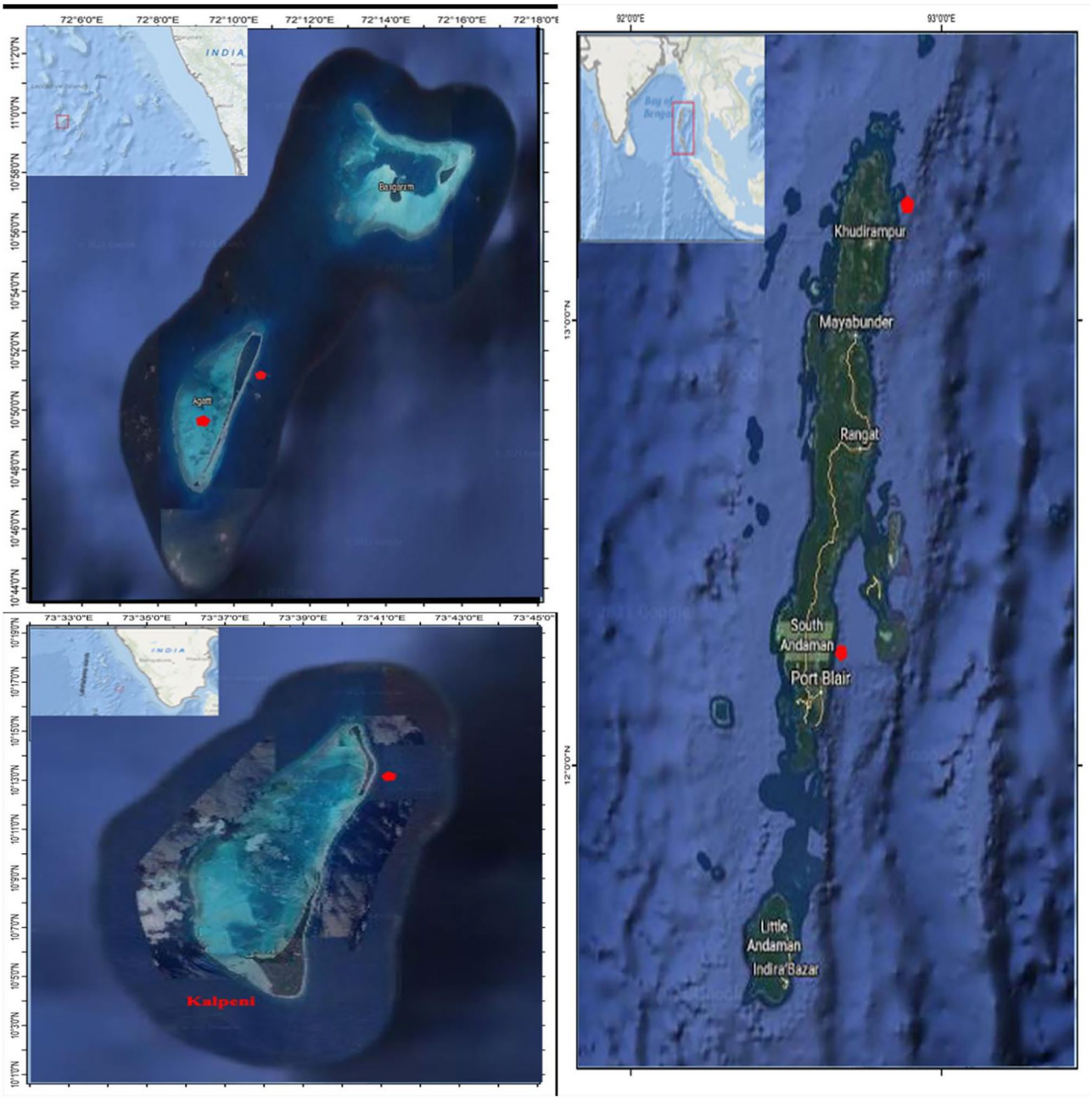

Fig. 1 Map of localities of Lakshadweep and Andaman and Nicobar Islands; red dots indicate the sampling locations

Sequencing and phylogenetic analysis

Amplified PCR products were sequenced using an ABI3730 capillary sequencer. Sequences were deposited in National Centre for Biotechnological Information (NCBI) data base and accession numbers are provided in Table 1. COI sequence (KJ550165) and 16S rRNA (KJ550812) sequence of the pacific specimen of *C. catus* were obtained from the NCBI data base. *Varioconus mercator* (MT240812, MT240813) was used as closely related outgroup and aligned with all sequences using the BioEdit (Hall, 1999). A maximum likelihood (ML) phylogenetic analysis with Kimura-2-parameters (K2P) model was performed using MEGA 7 (Kumar et al., 2016) to identify the group in which the *C. catus* specimens from different ecosystems are clustered.

Result

Morphological analysis

Morphological parameters were recorded for the *C. catus* species collected from different ecosystems rocky intertidal beds, lagoons, and inner reef platforms of Lakshadweep and Andaman Nicobar archipelagos of Indian Ocean. Shells are moderately medium sized ranging from 25 to 28 mm. Shell color varied across the ecosystems: olive-brown, blackish brown in rocky shores and orange in subtidal coral reef flats (Fig. 3). Relative diameter (RD) varies from 0.60 to 0.62 mm in rocky shores and 0.65 to 0.66 mm in subtidal coral reef flats. Position of maximum diameter (PMD) varies from 0.65 to 0.66 mm in rocky shores and 0.77 to 0.79 mm in subtidal coral reef flats, while the relative spiral height (RSH) measured 0.10 to 0.11 mm and 0.12 to 0.13 mm respectively.

Molecular analysis

Maximum likelihood (ML) phylogenetic clustering analysis tree was constructed for mitochondrial genes, protein coding COI (Fig. 2), and non-coding 16 s rRNA (Fig. 3) for the specimens of varying phenotypes of targeted species. The analysis revealed that the phenotypic variations in different environments are underpinned by the genome. Kimura 2 parameter (K2P) pairwise analysis–based estimated genetic variation in COI sequence in between rocky shore and coral reef ecotypes is 0.010 to 0.011 (1 to 1.1 percentage); on the contrary, rocky shore specimens of Lakshadweep and Andaman Islands showed genetic variation of 0.0 to 0.002 (0 to 0.02 percentage) and genetic distances between *C. catus* and *C. striolatus* are 0.108 to 0.112 (10.8. to 11.2 percentage) (Table 2). Mitochondrial 16SrRNA gene–based estimated evolutionary genetic divergences between the rocky and coral specimens of

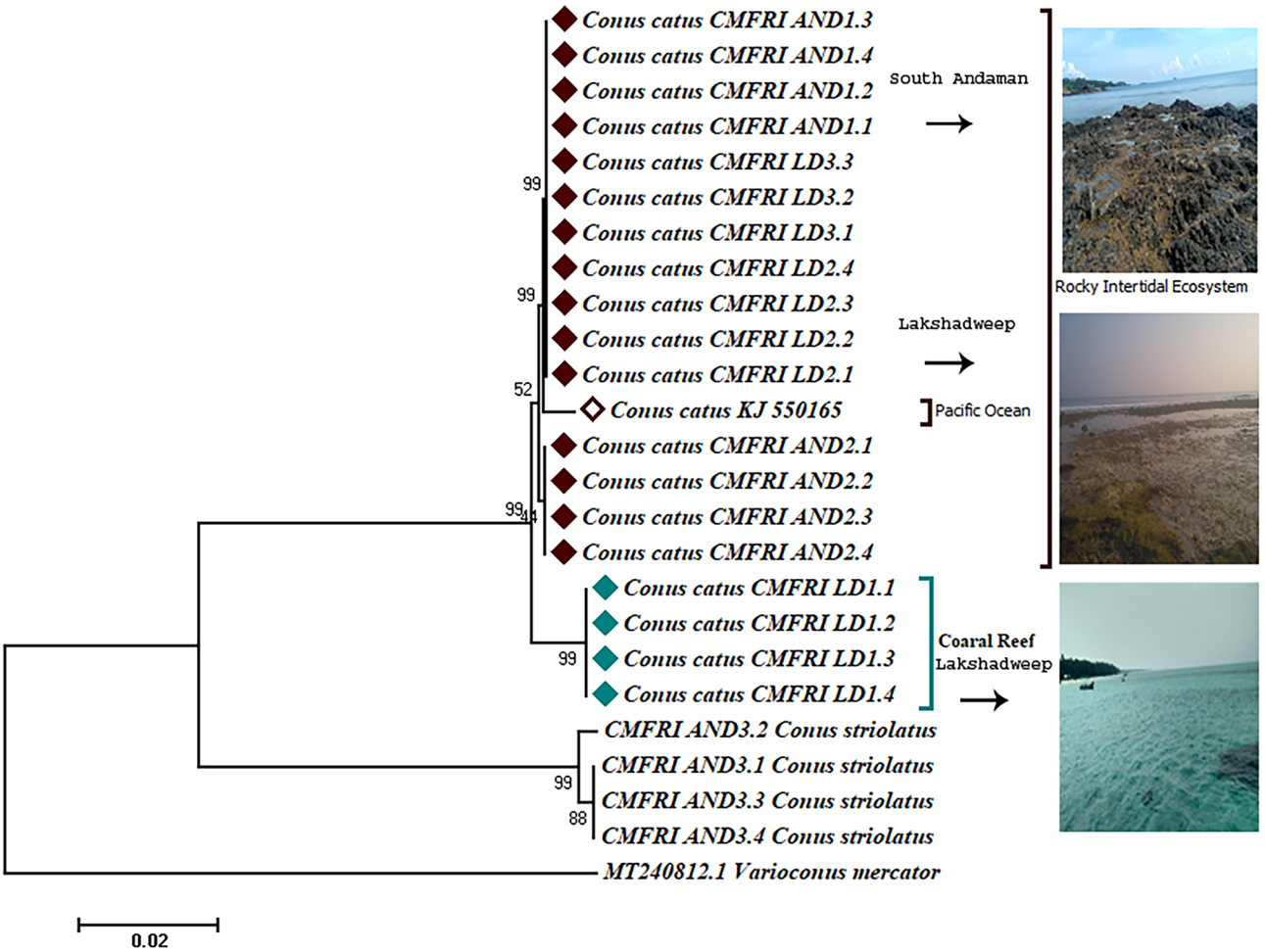

Fig. 2 Maximum likelihood phylogenetic tree based on partial mitochondrial COI gene sequences

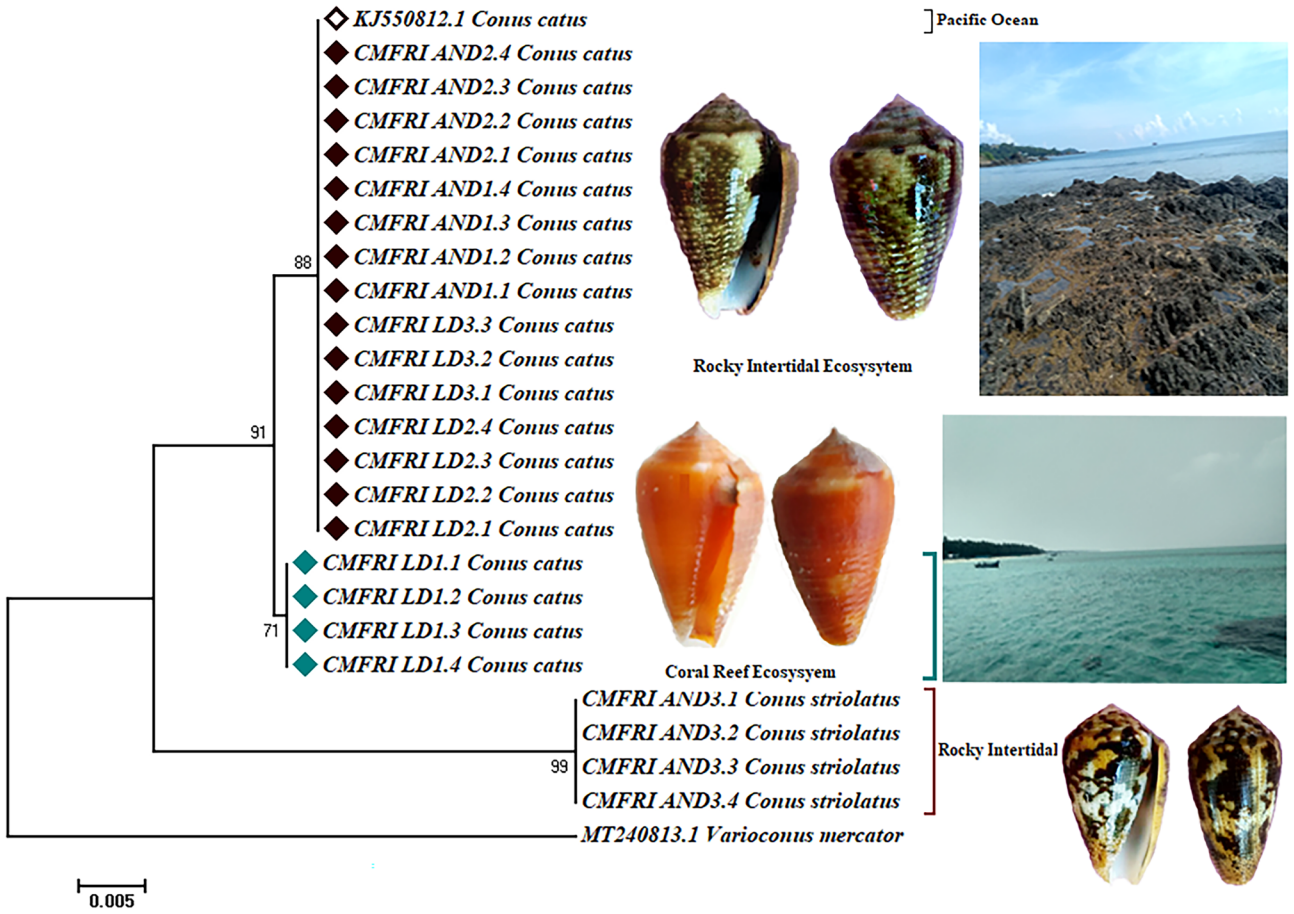

Fig. 3 Maximum likelihood phylogenetic tree based on partial mitochondrial 16S rRNA gene sequences. Images of rocky shore and coral reef ecophene of *C. catus* specimens and their habitats are provided

Conus catus are 0.004 (0.4 percentage), while those of rocky shore specimens of Lakshadweep and Andaman Islands are 0.0 (0 percentage) and genetic distances between *C. catus* and *C. striolatus* are 0.040 to 0.045 (4.0 to 4.5 percentage) (Table 3).

Among the 19 aligned sequences of *C. catus*, coral reef population has major base substitution pattern at six base pair locations of COI and two base pair locations of 16SrRNA revealing the genetic difference in coral reef specimens. Genetic diversity indices (detailed in Table 4) analyzed also confirmed population level divergence among coral and rocky groups. These COI sequences with 629 base pairs contained 622 monomorphic and seven polymorphic sites constituting three haplotypes. Coral reef haplotype included four individuals of coral reef population with six polymorphic sites. The 16S rRNA sequences with 465 base pairs contained two polymorphic sites constituting coral and rocky population haplotypes.

Discussion

Multiple mitochondrial molecular marker–based analysis on ecotypes of *Conus catus* revealed that ecological niche–dependent divergent evolutionary selective pressure is acting on both mitochondrial protein coding rapidly evolving COI marker and non-coding comparatively slowly evolving 16S rRNA but in different paces. We observed molluscan phylogeographical patterns that contradict expectations of low genetic connectivity between similar habitats separated by thousands of kilometers and high genetic connectivity in adjacent and ecologically distinct habitats. Similarly mitochondrial DNA sequence–based analysis in ecological speciation of reef fishes and shrimps with similar overlapping dispersal potential also contradicts expectation of large geographical isolation and giving new insights into the role of nearby ecological distinct habitat in speciation (Rocha et al., 2005; Wainwright et al., 2020).

Table 2 Kimura 2 parameter (K2P) model–based evolutionary genetic distance between COI sequences

Conus_catus_CMFRI_LD1.1																					
Conus_catus_CMFRI_LD1.2	**0.000**																				
Conus_catus_CMFRI_LD1.3	**0.000**	**0.000**																			
Conus_catus_CMFRI_LD1.4	**0.000**	**0.000**	**0.000**																		
Conus_catus_CMFRI_LD2.1	**0.010**	**0.010**	**0.010**	**0.010**																	
Conus_catus_CMFRI_LD2.2	**0.010**	**0.010**	**0.010**	**0.010**	0.000																
Conus_catus_CMFRI_LD2.3	**0.010**	**0.010**	**0.010**	**0.010**	0.000	0.000															
Conus_catus_CMFRI_LD2.4	**0.010**	**0.010**	**0.010**	**0.010**	0.000	0.000	0.000														
Conus_catus_CMFRI_LD3.1	**0.010**	**0.010**	**0.010**	**0.010**	0.000	0.000	0.000	0.000													
Conus_catus_CMFRI_LD3.2	**0.010**	**0.010**	**0.010**	**0.010**	0.000	0.000	0.000	0.000	0.000												
Conus_catus_CMFRI_LD3.3	**0.010**	**0.010**	**0.010**	**0.010**	0.000	0.000	0.000	0.000	0.000	0.000											
Conus_catus_CMFRI_AND1.1	**0.010**	**0.010**	**0.010**	**0.010**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**										
Conus_catus_CMFRI_AND1.2	**0.010**	**0.010**	**0.010**	**0.010**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**									
Conus_catus_CMFRI_AND1.3	**0.010**	**0.010**	**0.010**	**0.010**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**								
Conus_catus_CMFRI_AND1.4	**0.010**	**0.010**	**0.010**	**0.010**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**						
Conus_catus_CMFRI_AND2.1	**0.011**	**0.011**	**0.011**	**0.011**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**					
Conus_catus_CMFRI_AND2.2	**0.011**	**0.011**	**0.011**	**0.011**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.000**				
Conus_catus_CMFRI_AND2.3	**0.011**	**0.011**	**0.011**	**0.011**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.000**	**0.000**			
Conus_catus_CMFRI_AND2.4	**0.011**	**0.011**	**0.011**	**0.011**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.002**	**0.000**	**0.000**	**0.000**		
Conus_catus_KJ_550165	0.014	0.014	0.014	0.014	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006		
CMFRI_AND3.1_Conus_striolatus	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.106	0.106	0.106	0.106	0.110	

Table 2 (continued)

CMFRI_AND3.2_ Conus_striolatus	0.112	0.112	0.112	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.106	0.106	0.106	0.106	0.110	0.005		
CMFRI_AND3.3_ Conus_striolatus	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.106	0.106	0.106	0.106	0.110	0.000	0.005	
CMFRI_AND3.4_ Conus_striolatus	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.108	0.106	0.106	0.106	0.106	0.110	0.000	0.005	0.000

Previous studies suggest that adaptive divergence–dependent ecological speciation may happen despite opposition by gene flow and identifying the molecular basis of underlying genetic divergence with gene flow is a major task in evolutionary genetic studies (Schluter, 2001). Present study considered the archipelagic ecosystems in the different part of Indian Ocean as a natural laboratory and overcame the challenges like pelagic larval dispersal connectivity across the fluid medium which limits the understanding of the mechanism of ecological speciation (Palumbi, 1994; Feral, 2002). We compared the genetic variation of our rocky shore phenotype specimen with similar pacific specimen (Puillandre et al., 2014) and observed the same genetic connectivity across the rocky shore habituated specimens regardless of the sea and oceans they belong.

It is an already established fact that adaptive divergence is a key process in generating new species and it causes intraspecies genetic and phenotypic variations and may ultimately lead to ecological speciation (Schluter, 2001). This study gives new insight into molluscan speciation and provides the molecular and phenotypic evidence for parapatric speciation which may be caused by molluscan populations adapting to different ecological niches that vary across their ranges or accumulating different genetic mutations by selection or genetic drift (Coyne & Orr, 2004; Duda & Palumbi, 1999; Gavrilets, 2003).

C. striolatus a morphologically closely related species to rocky shore *C. catus* is the outcome of sympatric speciation through the selection pressures exerting on each other in rocky intertidal ecosystems. Expression of phenotypic traits may also be influenced by epigenetic factors like DNA methylation and histone modification (Vogt, 2017). Speciation of *C. catus* and *C. striolatus* is clear evidence for coevolution as well as sympatric speciation evolved in tandem due to selection pressures exerting on each other in rocky intertidal ecosystems. Alternate phenotypes of *C. catus* among the subtidal coral reef flats and rocky shores are due to the divergent selection–based adaptive radiation and parapatric selection pressure.

Table 3 Kimura 2 parameter (K2P) model–based evolutionary genetic distance between 16S rRNA sequences

CMFRI_LD1.1_Conus_catus																									
CMFRI_LD1.2_Conus_catus	**0.000**																								
CMFRI_LD1.3_Conus_catus	**0.000**	**0.000**																							
CMFRI_LD1.4_Conus_catus	**0.000**	**0.000**	**0.000**																						
CMFRI_LD2.1_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**																					
CMFRI_LD2.2_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	0.000																				
CMFRI_LD2.3_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	0.000	0.000																			
CMFRI_LD2.4_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	0.000	0.000	0.000																		
CMFRI_LD3.1_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	0.000	0.000	0.000	0.000																	
CMFRI_LD3.2_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	0.000	0.000	0.000	0.000	0.000																
CMFRI_LD3.3_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	0.000	0.000	0.000	0.000	0.000	0.000															
CMFRI_AND1.1_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**														
CMFRI_AND1.2_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**													
CMFRI_AND1.3_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**												
CMFRI_AND1.4_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**											
CMFRI_AND2.1_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**										
CMFRI_AND2.2_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**									
CMFRI_AND2.3_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**								
CMFRI_AND2.4_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**							
KJ550812.1_Conus_catus	**0.004**	**0.004**	**0.004**	**0.004**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**						
CMFRI_AND3.1_Conus_striolatus	0.040	0.040	0.040	0.040	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045					
CMFRI_AND3.2_Conus_striolatus	0.040	0.040	0.040	0.040	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.000				
CMFRI_AND3.3_Conus_striolatus	0.040	0.040	0.040	0.040	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.000	0.000			
CMFRI_AND3.4_Conus_striolatus	0.040	0.040	0.040	0.040	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.000	0.000	0.000		

Table 4 Details of genetic parameters inferred for *C. catus* haplotypes

Taxon	*Conus catus*	
	COI	**16S rRNA**
Number of sequences	19	19
Alignment length	629	465
Number of monomorphic sites	622	463
Number of polymorphic sites	7	2
Number of haplotypes	3	2
Haplotype diversity (Hd)	0.6082 ± 0.0895	0.3509 ± 0.1112
Nucleotide diversity (π)	0.003905 ± 0.002469	0.001509 ± 0.001330
Mean number of pairwise difference	2.456140 ± 1.389438	0.701754 ± 0.553398
Base frequency (%)	22.19%	32.95%
A	16.48%	14.36%
C	23.12%	21.25%
G	38.21%	31.44%
T		

Conclusion

The study concludes that parapatric speciation mechanism occurs when populations are partially isolated in adjacent ecologically different habitats like coral lagoons and rocky shores of archipelagic ecosystems. This ecological speciation process in *Conus catus* population may be caused by the adaptive radiations from coral reef ecological niche while accumulating different mutations. Conventional allopatric speciation models cannot account for all tropical archipelagic invertebrate biodiversity because it requires geographical isolation between species, a condition difficult to meet in an Island ecosystem where most marine invertebrates have a pelagic dispersing larval phase. We contradict the expectations of low genetic connectivity between similar habitats separated by thousands of kilometers and high genetic connectivity in adjacent and ecologically distinct habitats in molluscan phylogeographic studies. The study revealed that ecotypes of *C. catus* under natural selection in alternative habitat at adjacent geographical locations override gene flow, and drive the semi isolated populations along separate evolutionary pathways. It also establishes and confirms the role of ecologically different habitats in speciation and biodiversity conservation.

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Data availability All data generated or analyzed during this study are included in this published article, and the sequence data have been deposited in GenBank, NCBI. Any other information regarding the current study is available from the corresponding author on reasonable request.

Declarations

Ethical approval All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements.

Conflict of interest The authors declare no competing interests.

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A new species of cone snail, *Conus laccadivensis* sp. nov.: (Gastropoda: Conidae) from the Islands of Lakshadweep Archipelago

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ABSTRACT

The present paper describes the cone snail, *Conus laccadivensis* sp. nov., based on the morphological and molecular characters of the materials collected from Kalpeni and Androth Islands of Lakshadweep Archipelago. The species closely resembles the tropical species *Conus distans* (Hwass in Bruguière (1792) which is widely distributed throughout the Indo-Pacific regions. Morphologically *C laccadivensis* sp. nov., differs from *C. distans* in shell colour and spire characteristics. *Conus laccadivensis* sp. nov., possess distinct colour patterns with large and closely spaced coronations. The maximum likelihood clustering approaches using the mitochondrial genes such as COI, 16S rRNA and 12S rRNA revealed a separate genetic cluster confirming the newly identified species.

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1. Introduction

The Lakshadweep Archipelago in the Indian Ocean consists of a wide range of ecosystems namely coral reefs, mangroves, sea grasses, and intertidal rocky shores. These niches harbour diverse species, including molluscan fauna (Ravinesh and Kumar, 2015) with a wide range of archipelagic endemism. The marine species undergoes genetic changes through accumulation of mutations and genetic drifts which are subjected to selection pressure (Palumbi, 1994).

The family Conidae, consists of more than 900 described species (MolluscaBase, 2020) distributed across intertidal zones to deep waters (Tenorio et al., 2020). The cone snails are much valued by the shell collectors, conchologists and malacologists for their striking forms, colours and ecological patterns (Rice, 2007). Cone snails are of significant importance to the marine biome due to their exceptional species richness (Röckel et al., 1995; Ravinesh et al., 2018). Cone snails are of special pharmaceutical importance with 200 different conopeptides from the conotoxins, which are deployed during capture of prey (Livett et al., 2006; Olivera et al., 1999; Dutertre et al., 2013). Molecular information is widely used in combination with morphological characters for taxonomic classification and is become the confirmative tool for species delimitation (Duda and Kohn, 2005; Lawler and Duda, 2017).

A total of 78 cone snail species were recorded from Lakshadweep Archipelago (Smith, 1906; Hornell, 1921; Nagabhushanam and Rao, 1972; Appukuttan et al., 1989; Rao and Rao, 1991; Apte, 1998, 2014; Rao, 2003; Ravinesh and Kumar, 2015). Recently, Ravinesh et al. (2018) documented 48 species of cone snails from the Islands of Lakshadweep. The specimens in the present study were collected from the inhabited islands of Lakshadweep and the *Conus laccadivensis* sp. nov., was compared with the closely related species, *C. distans* by both the morphology and genetic parameters.

2. Materials & methods

Abbreviations used

Central Marine Fisheries Research Institute: CMFRI; Muséum National D'histoire Naturelle: MNHN; Shell Length: SL; Shell Width: SW; Aperture Height: AH: Relative diameter: RD; Relative spire height: RSH; Position of the maximum diameter: PMD; National Center for Biotechnology Information: NCBI.

Sample collection

Live specimens were collected from the intertidal benches and coarse sand (Fig. 1. 1–2) of Kalpeni (n=4) and Andrott (n=1) Islands along the Lakshadweep archipelago (Fig. 2). All the specimens were collected by snorkelling or SCUBA. Tissue samples of muscular footormantle were removed and preserved in 95% ethanol until further use. The description of the shell morphology was carried out following Röckel et al. (1995).

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Fig. 1. Live specimens of *Conus laccadivensis* sp. nov., from the intertidal benches, coarse sand intertidal region of Kalpeni Island (1–2).

DNA extraction and PCR amplification

Genomic DNA was isolated from foot tissue using customized Origin marine animal kit, by following manufacturer's instructions. The quality and quantity of DNA were tested by Nanodrop (Eppendorf Pvt. Ltd). Fragments of the mitochondrial gene 12S rRNA, 16S rRNA and cytochrome oxidase subunit I (COI) gene were amplified employing universal primers 12S1/12SB (Simon and Franke, 1991) 16Sar/16Sbr (Palumbi, 1996) and LCO1490/HCO2198 (Folmer et al., 1994), respectively. All the PCR reactions were performed in 25 μl, containing 75 ng of DNA, 2.5 mM $MgCl_2$, 0.3 mM of each primer. Amplification reactions consisted of an initial denaturation step at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing for 40 s at 54 °C, 52 °C, 50 °C for 12S rRNA, 16S rRNA and COI (respectively), and the extension occurred at 72 °C for 1 min. The final extension was at 72 °C for 5 min. Amplified PCR products were visualized in 1.2% agarose gels containing ethidium bromide.

Sequencing and phylogenetic analysis

Amplified PCR products were sequenced using an ABI3730 capillary sequencer and sequences were obtained. Further, COI, 16S rRNA and 12S rRNA sequences from the closely related species were obtained from the GenBank, NCBI database (Table 1) and aligned with the newly generated sequences using the BIO Edit (Hall, 1999).

A maximum likelihood (ML) analysis with Kimura-2-parameters (K2P) model was performed using MEGA 7 (Kumar et al., 2016) to identify the group in which the new species is clustered. The ML tree was constructed with closely related sequences, corresponding to the species belonging to the genus *Conus (Fraterconus)* as defined by Puillandre et al. (2014), with the more distant species forming the out groups.

Results:

Five specimens of *Conus laccadivensis* sp. nov., were examined for morpho-meristic characters and successfully sequenced for fragments of the COI, 12S and 16S rRNA genes.

Systematics

Superfamily **Conoidea** Fleming 1822
Family **Conidae** Fleming 1822
Genus ***Conus*** Linnaeus, 1758
***Conus laccadivensis* sp. nov.,**

Conus distans Hwass in Bruguière, 1792: Rao (2003) 314. Pl. 64, Fig. 5–7.

Conus distans Hwass in Bruguière; Ravinesh, Biju Kumar and Kohn (2018) 478; Fig. 4. A–C, D–F

Type material:

Holotype and 4 paratypes. The holotype and paratype deposited in the national repository Museum collections of Marine Biodiversity Museum, Central Marine Fisheries Research Institute, (holotype accession number DB.35.1.26) Cochin, Kerala, India.

Type Locality: Kalpeni Island, Lakshadweep archipelago, Arabian Sea, Central Indian Ocean, Location: 10.07^0 25'72" N; 73.63^0 55' 18" E.

Etymology: This new species of *Conus* is named based on the type locality Lakshadweep, Arabian Sea of India (formerly known as Laccadives). The Lakshadweep Archipelago is a unique coral reef Island ecosystem comprised of coral reefs, seagrasses, mangroves and intertidal rocky shores and serves as a hub for a wide variety of molluscan fauna.

Distribution and habitat:

Conus laccadivensis sp. nov., is so far only known from the Kalpeni and Andrott Islands of the Lakshadweep Archipelago. It is distributed in the shallow coral reefs in coarse sand or coarse rubble. This species occurs in the shallow areas of coral reefs up to 10 m.

Holotype: CMFRI MBD C2.1 (Fig. 3. 3–4) Standard Length 109 mm; Shell Width 63 mm; Aperture Height 94 mm; Relative diameter 0.062 mm; Relative spire height 0.12 mm; Position of the maximum diameter 0.88 mm; Kalpeni Island of Lakshadweep archipelago, Arabian Sea, Central Indian Ocean, Location: 10.07^0 25'72" N; 73.63^0 55' 18" E.

Paratypes: CMFRI MBD C2.2 Standard Length 90 mm; Shell Width 56 mm; Aperture Height 78 mm; Relative diameter 0.061 mm; Relative spire height 0.11 mm; Position of the maximum diameter 0.87 mm; Kalpeni Island of Lakshadweep archipelago, Arabian Sea, Central Indian Ocean, Location: 10.07^0 25'72" N; 73.63^0 55' 18" E.

CMFRI MBD C2.3 Standard Length 79 mm; Shell Width 50 mm; Aperture Height 68 mm; Relative diameter 0.059 mm; Relative spire height 0.12 mm; Position of the maximum diameter 0.85 mm; Kalpeni Island of Lakshadweep archipelago, Arabian Sea, Central Indian Ocean, Location: 10.07^0 25'72" N; 73.63^0 55' 18" E.

CMFRI MBD C2.4 Standard Length 91 mm; Shell Width 56 mm; Aperture Height 78 mm; Relative diameter 0.061 mm; Relative spire height 0.11 mm; Position of the maximum diameter 0.88 mm; Adroth Island of Lakshadweep archipelago, Arabian Sea, Central Indian Ocean, Location: 10.80^0 89'87" N; 73.66 04'30" E

CMFRI MBD C2.5 Standard Length 104 mm; Shell Width 56 mm; Aperture Height 89 mm; Relative diameter 0.062 mm; Relative spire height 0.11 mm; Position of the maximum diameter

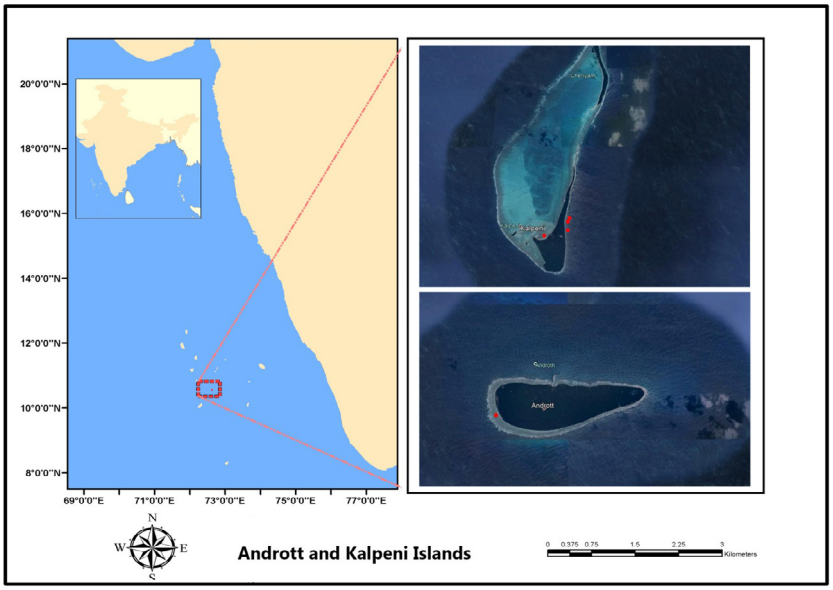

Fig. 2. Andrott and Kalpeni Islands of Lakshadweep archipelago. Red dots indicate the species occurrence.

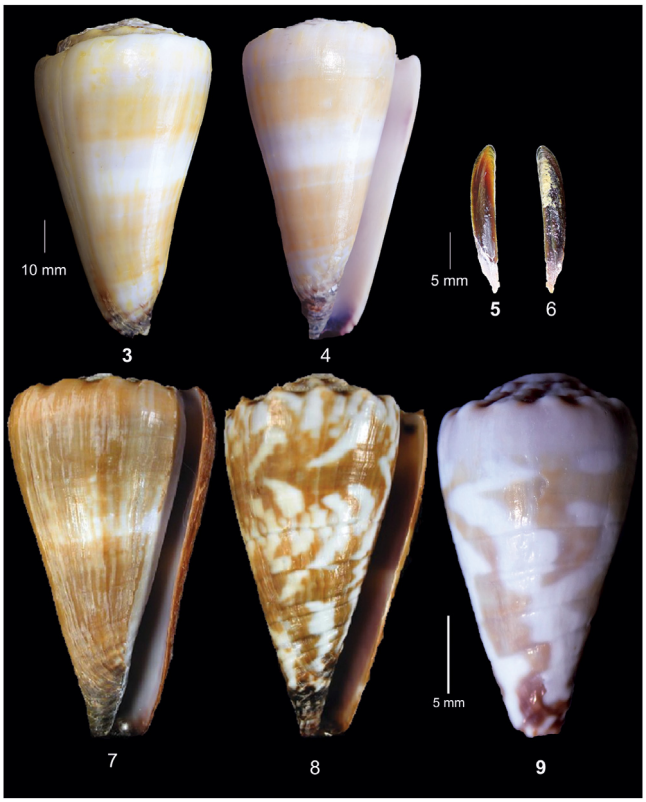

Fig. 3. Holotype specimen of *Conus laccadivensis* sp. nov., CMFRI MBD C2.1 *(3–4),* Operculum of *C. laccadivensis* (5–6). *Conus distans* Hwass, 1792 MNHN-IM-2007-30646 (7), juvenile specimen of *C distans* MNHN-IM-2007-30833 (8), juvenile specimen of *Conus laccadivensis* sp. nov., (9).

0.88 mm; Kalpeni Island of Lakshadweep archipelago, Arabian Sea, Central Indian Ocean, Location: 10.07^0 25'72'' N; 73.63^0 55' 18'' E.

Additional material: *Conus distans* Hwass, 1792 MNHN-IM-2007-30646 Standard Length 70.2 mm (Fig. 3. 7) and MNHN-IM-2007-30833 Standard Length 46.7 mm (Fig. 3. 8)

The authority for the new species *Conus Laccadivensis* is here specifically identified as Laxmilatha and Shijin.

3. Description

The shell moderately large ranging from (79–109 mm); solid and heavy. The shell 10 to 13 whorls; spire low (RSH: 0.11–0.12 mm).; protoconch is broken in all the examined specimens. Outline convex to strongly convex near shoulder, straight below. Shoulder broad, angulated, with heavy rounded coronations; coronation large widely spaced, rounded. Last whorl conical (RD:

Table 1
Specimens used for phylogenetic analyses.

Species	Specimen voucher No. (Location)	NCBI Accession No.		
		COI	16S	12S
Conus laccadivensis sp. nov., (Haplotype 1)	CMFRI MBD C2.1 (Kalpeni, Arabian Sea)	MH571764	MH571762	MH571760
Conus laccadivensis sp. nov., (Haplotype 2)	CMFRI MBD C2.4 (Androth, Arabian Sea)	MH571765	MH571763	MH571761
Conus distans	MNHN-IM-2007-30833 (Vanuata, Pacific)	KJ550205.1	KJ550822.1	KJ 551520.1
Conus distans	UF 295249 (Indo West-Pacific)	KJ549897.1	KJ550604.1	KJ551286.1
Conus distans	MW 607 (Unknown)	KJ606037.0	KJ606035.1	KJ606033.1
Conus chiangi	–	GU134381.1	GU134361.1	GU134364.1
Conus zonatus	–	GU134383.1	GU134362.1	GU134366.1
Conus mappa	–	KJ549949.1	KJ550666.1	KJ551052.1
Conus curassaviensis	–	KJ549893.1	KJ550595.1	KJ550963.1
Conus lenavati	–	KJ550324.1	KJ550647.1	KJ551124.1
Conus roseorapum	–	KJ550544.1	KJ550945.1	KJ551230.1
Conus queenslandis	–	KJ550432.1	KJ550897.1	KJ551188.1
Californiconus californicus	–	KJ549878.1	KJ550576.1	KJ550992.1

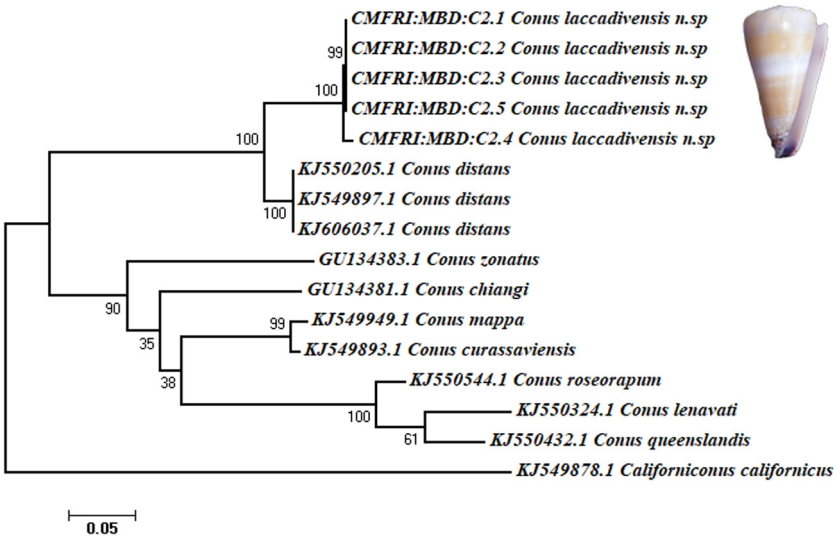

Fig. 4. Maximum Likelihood (ML) phylogenetic tree obtained with mitochondrial COI gene.

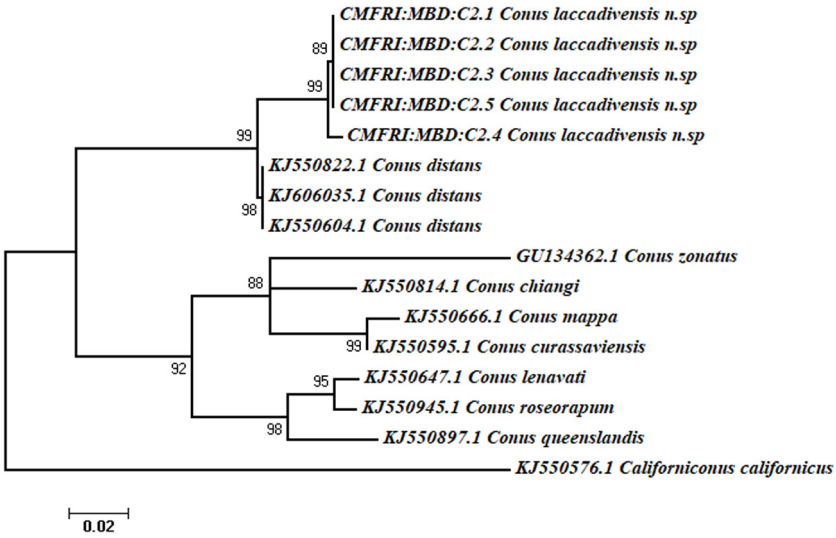

Fig. 5. Maximum Likelihood (ML) phylogenetic tree obtained with the combined 16S and 12S rRNA genes.

0.59–0.61 mm) with position of maximum diameter (PMD) ranging from 0.85 to 0.87 mm. Aperture moderately narrow, slightly extended anteriorly, outer lip sharp, concave at the middle. Columella short, narrow. Shell light grey in colour with a prominent white middle band closely below centre and at shoulder. Mouth dark violet or blackish violet. Periostracum is brown, thick and rough. Operculum horny, 20.6 mm length and width 3.8 mm (Fig. 3. 5–6).

Table 2
Estimates of evolutionary divergence between partial mitochondrial COI sequences based on the Kimura 2 parameter.

CMFRI:MBD:C2.1_Conus laccadivensis **sp. nov.,**														
CMFRI:MBD:C2.2_Conus laccadivensis **sp. nov.,**	0.000													
CMFRI:MBD:C2.3_Conus laccadivensis **sp. nov.,**	0.000	0.000												
CMFRI:MBD:C2.4_Conus laccadivensis **sp. nov.,**	0.008	0.008	0.008											
CMFRI:MBD:C2.5_Conus laccadivensis **sp. nov.,**	0.000	0.000	0.000	0.008										
KJ550205.1_Conus_distans	**0.070**	**0.070**	**0.070**	**0.076**	**0.070**									
KJ549897.1_Conus_distans	**0.070**	**0.070**	**0.070**	**0.076**	**0.070**	0.000								
KJ606037.1_Conus_distans	**0.070**	**0.070**	**0.070**	**0.076**	**0.070**	0.000	0.000							
GU134381.1_Conus_chiangi	0.181	0.181	0.181	0.187	0.181	0.194	0.194	0.194						
GU134383.1_Conus_zonatus	0.205	0.205	0.205	0.205	0.205	0.182	0.182	0.182	0.163					
KJ549949.1_Conus_mappa	0.203	0.203	0.203	0.207	0.203	0.199	0.199	0.199	0.147	0.167				
KJ549893.1_Conus_curassaviensis	0.209	0.209	0.209	0.214	0.209	0.205	0.205	0.205	0.147	0.162	0.017			
KJ550324.1_Conus_lenavati	0.213	0.213	0.213	0.213	0.213	0.192	0.192	0.192	0.193	0.201	0.170	0.170		
KJ550544.1_Conus_roseorapum	0.202	0.202	0.202	0.206	0.202	0.194	0.194	0.194	0.155	0.192	0.154	0.146	0.099	
KJ550432.1_Conus_queenslandis	0.237	0.237	0.237	0.241	0.237	0.217	0.217	0.217	0.191	0.196	0.180	0.176	0.090	0.087

Table 3
Estimates of evolutionary divergence between partial mitochondrial 16S and 12s rRNA sequences based on the Kimura 2 parameter.

CMFRI:MBD:C2.1_ *Conus laccadivensis* **sp. nov.,**														
CMFRI:MBD:C2.2_ *Conus laccadivensis* **sp. nov.,**	0.000													
CMFRI:MBD:C2.3_ *Conus laccadivensis* **sp. nov.,**	0.000	0.000												
CMFRI:MBD:C2.5_ *Conus laccadivensis* **sp. nov.,**	0.000	0.000	0.000											
CMFRI:MBD:C2.4_ *Conus laccadivensis* **sp. nov.,**	0.006	0.006	0.006	0.006										
KJ550822.1_Conus_distans	**0.026**	**0.026**	**0.026**	**0.026**	**0.028**									
KJ606035.1_Conus_distans	**0.026**	**0.026**	**0.026**	**0.026**	**0.028**	0.000								
KJ550604.1_Conus_distans	**0.026**	**0.026**	**0.026**	**0.026**	**0.028**	0.000	0.000							
KJ550814.1_Conus_chiangi	0.117	0.117	0.117	0.117	0.124	0.109	0.109	0.109						
GU134362.1_Conus_zonatus	0.142	0.142	0.142	0.142	0.150	0.137	0.137	0.137	0.080					
KJ550666.1_Conus_mappa	0.124	0.124	0.124	0.124	0.129	0.112	0.112	0.112	0.059	0.090				
KJ550595.1_Conus_curassaviensis	0.114	0.114	0.114	0.114	0.119	0.102	0.102	0.102	0.053	0.088	0.011			
KJ550647.1_Conus_lenavati	0.126	0.126	0.126	0.126	0.129	0.111	0.111	0.111	0.090	0.105	0.091	0.083		
KJ550945.1_Conus_roseorapum	0.121	0.121	0.121	0.121	0.124	0.109	0.109	0.109	0.093	0.110	0.090	0.083	0.015	
KJ550897.1_Conus_queenslandis	0.121	0.121	0.121	0.121	0.124	0.109	0.109	0.109	0.095	0.122	0.103	0.095	0.048	0.046

4. Remarks

Conus laccadivensis sp. nov., is morphologically similar to the Pacific species *C. distans but* with a very distinct colour pattern and with large and closely spaced coronations. The variation of the irregular oblique markings and colour pattern are also distinct in juvenile stages. Colour of the shell is greenish tan with a few white spots on the mid-body and above the base. In the previous findings, these younger specimens of *C. distans* were described several times as distinct species: *C. kenyonae* (Brazier, 1896), originally described from Shark's Bay Western Australia, and its variety *arrowsmithensis* (described from Marshall Islands, *C. waterhousae* (Brazier, 1896), described from Solomon Islands and *C. chinoi* (Shikama, 1970) from Japan.

5. Molecular analyses

The average sequence length of COI (658 bp), 16S (500 bp) and 12S (450 bp) were obtained from the five specimens of *Conus laccadivensis* sp. nov., and haplotypes were submitted to the NCBI(Table 1) .

Analyses of the partial mitochondrial COI sequence revealed well differentiated clades for C. *laccadivensis* sp. nov., and *C. distans* (Fig. 4), partial 16S rRNA and 12S rRNA gene sequences resulted in phylogenetic trees with identical topology (Fig. 5). The intra-species K2P genetic difference among the five specimens of *Conus laccadivensis* sp. nov., ranged from 0 to 0.7% for COI and 0 to 0.2% for 16S and 12S rRNA genes, whereas inter-species difference with *Conus distans* was 7 to 7.6% for COI (Table 2) and 2.5 to 2.8% in 12S rRNA and 16S rRNA (Table 3) genes which is more than the threshold limit for inter-species genetic distances (> 6% in COI and > 2% in 16S, 12SrRNA) in Conidae (Puillandre et al., 2014).

6. Discussion

Five specimens of the cone snails, collected from the Andrott and Kalpeni Islands of Lakshadweep Archipelago, were identified as a new species based on morphometry and molecular analyses. The molecular analyses of COI, 16S rRNA and 12S rRNA confirmed *C. laccadivensis* sp. nov., as a newly identified species. The species morphologically resembles *C. distans,* which is distributed in the Pacific Ocean (Puillandre et al., 2014).

The congruence of the phylogenetic trees resulting from the three unlinked mitochondrial markers (COI, 16S rRNA, 12S rRNA) with identical topology as *C. laccadivensis* sp. nov., belonged to a single clade and the sequences of *C. distans* from GenBank clustered in a separate supportive clade. Phylogenetic analyses clearly indicated *C. laccadivensis* sp. nov., and *C. distans* are sister groups, belonging to the clade *Fraterconus* which was previously defined by Puillandre et al. (2014) with a single species *C. distans. Fraterconus* was a sister group for all other Conus species which represent around 85 percentage of conidae family (Puillandre et al., 2014).

The *p*-distance of all the three genes between *C. laccadivensis* sp. nov., and *C. distans* was more than the threshold genetic distances generally considered as interspecific distances used for species delimitation and validation in this hyperdiverse cone snails (Duda et al., 2008; Puillandre et al., 2011, 2014; Abalde et al., 2017).

It is strong, although indirect, evidence for reproductive isolation among, *C. distans* and for *C. laccadivensis* sp. nov., both of which are characterized by occurrence in highly divergent geographic regions. All these geographic and genetic factors indicate strongly that *C. distans* and *C. laccadivensis* sp. nov., are isolated species as the geographic distributions of the two species are clearly allopatric.

Ecological adaptive radiation based approaches have revealed that divergent venoms are evolving in cone snails with speciation

and a novel species might produce modified peptides (Duda, 2008; Duda and Palumbi, 1999). Speciation is promoted by divergent natural selection in many taxa including *Conus* (Kohn, 1990; Palumbi, 1994; Duda and Palumbi, 1999). However, geographic and genetic factors have been affecting the process of speciation (Palumbi, 1994). Genetic analysis of rapidly evolving mitochondrial coding (COI) genetic markers and slowly evolving non-coding (12S rRNA, 16S rRNA) genetic markers are thus an ideal tools for understanding the evolutionary mechanism of speciation.

Here, we describe a new species of cone snail, *Conus laccadivensis* sp. nov., from the Lakshadweep Archipelago. The partial sequencing of mitochondrial COI, 16s rRNA and 12s rRNA genes and the unique shell characteristics, substantiating the validity of the newly identified species. The present study highlights the identification and description of a new species of cone snail from the coral reef ecosystem of Lakshadweep Archipelago, which in turn has important implications for natural resource protection, conservation and management of cone snails.

CRediT authorship contribution statement

P. Laxmilatha: Conception and design of study, Writing - original draft, Writing - review & editing. **Shijin Ameri:** Conception and design of study, Analysis and/or interpretation of data, Writing - original draft, Writing - review & editing. **K.A. Labeeb:** Analysis and/or interpretation of data. **L. Ranjith:** Writing - review & editing. **A. Kathirvelpandian:** Analysis and/or interpretation of data.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

All data generated or analysed during this study are included in this published article. Any other information's regarding the current study is available from the corresponding author on reasonable request

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ABSTRACT PROCEEDINGS

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Department of Marine Biology, Microbiology and Biochemistry
School of Marine Sciences
Cochin University of Science and Technology

BMB-P-03

INTEGRATED TAXONOMY REVEALS THE EXCEPTIONAL PHENOTYPIC PLASTICITY IN *CONUS (PINOCONUS) CATUS* AMONG THE ANDAMAN AND LAKSHADWEEP ARCHIPELAGOS

Shijin Ameri[1], Laxmilatha Pappurajam[1], Labeeb, K.A[1]. Ranjith, L.[1] and Kathirvel Pandian, A.[2]

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Species delimitation in the cone snails has long been difficult, because of its extraordinary taxonomic and ecological diversity. Shell characters and primarily colour patterns are the basic taxonomic features. Here we used molecular sequence data from three mitochondrial gene regions (16S rRNA, 12SrRNA and cytochrome oxidase subunit I) and a nuclear gene (Histone3) to resolve phenotypic plasticity among the *Conus (Pinoconus) catus* in varying ecosystems of Andaman and Lakshadweep archipelagos.Morphological parameters were recorded for the phenotypes of the species collected from different ecosystems such as rocky intertidal beds, lagoons, inner reef platforms, sea grass beds, and sandy shores. Specific differences were observed for shell coloration. Maximum likelihood clustering approach were done based on mitochondrial genes, COI (658bp), 16s rRNA (540bp), 12s rRNA (460bp) and Nuclear H3 gene (360bp) amplified in the specimens of varying phenotypes of *C.catus*. The analysis revealed that *C.catus* showsexceptional phenotypic plasticity among ecosystems without any genetic modification. This current plasticity could be the outcome of epigenetic factors associated with ecological adaptation. The present study generated species-specific molecular signatures from varying phenotypes of *C.catus* and closely related species *Conus striolatus*. These findings provide confirmation of phenotypic variation and can be used effectively for conotoxin based bio prospecting research on the species and also conservation and management purposes.

Keywords: Conus, COI, 16SrRNA, 12SrRNA, Histone3, Indian Ocean.

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He / She also presented ~~a paper~~ / presented a poster / ~~delivered a Invited talk~~ / ~~chaired a techincal session~~ titled INTEGRATED TAXONOMY REVEALS THE EXCEPTIONAL PHENOTYPIC PLASTICITY IN CONUS (PINOCONUS) CATUS AMONGT THE ANDAMAN AND LAKSHDWEEP ARCHIPELAGOS.

We wish his / her the best for future endeavours.

Dr. Priyaja P.
Assistant Professor
Department of Marine Biology,
Microbiology and Biochemistry
School of Marine Sciences, CUSAT
& Organising Secretary ICB 2019

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12 March 2020

To Sir or Madam:

This letter confirms that Shijin Ameri presented a paper titled, "Integrative Taxonomy of Cones Snails (Conidae, Gastropoda) Giving new Insight Into The Role of Ecosystem in Speciation", during a(n) Oral session on 17 February, 2020 at the Ocean Sciences Meeting 2020. The Ocean Sciences Meeting 2020 took place 16-21 February, 2020 at the San Diego Convention Center in San Diego, CA, USA.

Should you have any questions, please contact the AGU Meetings office at abstracts@agu.org.

Sincerely,

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Integrative taxonomy reveals that all the phenotype variations are not underpinned by genome in Conidae (Gastropoda, Mollusca)

Laxmilatha Pappurajam[1], Shijin Ameri[1], K A Labeeb[1], A Kathirvel Pandian[2], L Ranjith[1] and A Gopalakrishnan[1]

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The cone snails are hyper diverse group of marine gastropods and the shell morphology is the base of primary species identification. Among the Conidae, homoplasy and plasticity of morphological characters are common. Since speciation is positively correlated with peptide complexity in Conidae family, the present study was carried out to ascertain the genetic status of drastically varying phenotypes observed among the targeted Conus species of different environments and to detect cryptic species using mitochondrial and nuclear markers. Morphological parameters were recorded for the phenotypes of the species. Specific differences were observed for shell coloration. Three mitochondrial genes, COI (658bp), 16s rRNA (550bp), 12s rRNA (450bp) and Nuclear H3 gene (350bp) were amplified in five specimens each of varying phenotypes of targeted species. The analysis revealed that the phenotypic variations in *C. inscriptus C.amadis and C.malacanus* in different environments are not underpinned by the genome, The present study generated species-specific molecular signatures from varying phenotypes of conidae Since speciation is positively correlated with the complexity of conopeptides of the species, the findings provide confirmation of phenotypic variation and will be used for bioprospecting research on the species and also conservation and management of the species in Indian waters.

Gmail **Shijin A <shijin2050@gmail.com>**

Ocean Sciences Meeting Early Career Scientist Travel Grant Notification

1 message

Carissa Lundquist <clundquist@agu.org> 12 November 2019 at 01:50
To: "shijin2050@gmail.com" <shijin2050@gmail.com>

11 November 2019

Shijin Ameri:

Congratulations! You have been selected to receive an Early Career Scientist Travel Grant to attend the 2020 Ocean Sciences Meeting, 16-21 February 2020 in San Diego, California, U.S.A where you will present your abstract titled “Integrative taxonomy of cones snails (Conidae, Gastropoda) giving a new insight into the role of ecosystem in speciation.” Your travel grant is in the amount of $1000.00 USD. This email will serve as your official award notification.

To accept your award, you must log into the AGU Grants & Scholarships Site and complete the acceptance form **by 27 November 2019, 11:59 P.M. ET**. Banking information is required as part of your acceptance, as well as U.S. visa appointment confirmation (if applicable). If you do not formally accept by the deadline, AGU will assume that you decline this award, and the grant funds will be reallocated.

To access the form, click on “My Account” and then “Needs Attention” or “In Progress”. This email will serve as your official award notification, so please print/save this email and the acceptance form for your records.

Congratulations again on receiving this award. We look forward to meeting you at the Ocean Sciences Meeting. Feel free to reach out with any questions or concerns.

Please contact the AGU Grants Team at grants@agu.org with any questions regarding your award.

Regards,

Carissa Lundquist
Student Grants and Scholarships Coordinator

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OFFICIAL MEMORANDUM

Sub: Registration for Ph.D. Degree Programme in **Biosciences.**

Ref: 1) Regulation Governing the Degree of Ph.D. Notification No. MU/ACC/CR.9/2009-10/A2, Dated 03.09.2010 and Circular No. MU/ACC/CR.49/2015-16/A2 dated 21.05.2016 relating to guidelines for implementing the regulation.
2) Provisional admission O.M. No.MU/ACC/Ph.D./CR.51/2016-17/ A3 Dated: 15.05.2017 of the Registrar, Mangalore University.
3) Letter No.MU/ACC/Ph.D-Reg./CR.194/2016-17/A3, Dated: 06.10.2018 of the Registrar, Mangalore University.

*****

The application of **Mr. Shijin Ameri, Research Scholar in Biosciences, ICAR – Central Marine Fisheries Research Institute, Ernakulam P.O., Kochi - 682018** has been accepted for registration for Ph.D. programme of this University with effect from **22.02.2017.** He is permitted to work and prepare a thesis in **"Biosciences"** on the topic entitled **"INTEGRATED TAXONOMY AND MOLECULAR PHYLOGENY OF CONIDAE (MOLLUSCA: GASTROPODA) OF THE INDIAN COAST"** under the guidance of **Dr. P. Laxmilatha, Principal Scientist, ICAR – Central Marine Fisheries Research Institute, Ernakulam P.O., Kochi - 682018** subject to the following conditions:

1. The candidate shall undertake the research work on **FULL– TIME** basis. He shall work in **ICAR – Central Marine Fisheries Research Institute, Ernakulam P.O., Kochi - 682018.**
2. The candidate is not permitted to join or register for any other degree programme of this University or any other University during the period of registration.
3. The title of the thesis can be changed or modified/altered with the prior approval of the competent authority of Mangalore University.
4. The candidate shall pay annual / term fee as fixed by the University from time to time along with half yearly progress report.
5. a) The candidate shall submit half yearly progress report regularly to the Registrar (Evaluation) through the guide and Chairperson of the Department/Head of the Institution during June and December.

   b) Progress report may contain new data generated or techniques/concepts developed /standardization in research methodology/discussion of the work done etc.
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